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(54) Title: DEVICES AND METHODS FOR RECOVERY OF A TARGET MOLECULE FROM A SAMPLE

(57) Abstract: The invention relates to a device for recovering a target molecule from a biological sample. The device includes a first unit defining a chamber, and the chamber includes a first region for receiving a sample and a second region including at least one electrophoretic medium. The device includes a second unit extending from a first end to a second end, and the second unit is interfaceable with the first unit. A mesh structure is located at the second end of the second unit and is impregnated with an electrophoretic medium that includes copolymerized capture probes. The second region is located between the first region and the mesh structure when the first unit is interfaced with the second unit.

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# DEVICES AND METHODS FOR RECOVERY OF A TARGET MOLECULE FROM A SAMPLE

## CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of and priority to United States provisional patent application serial number 60/440,500, filed January 16, 2003, the entirety of which is incorporated herein by reference.

## TECHNICAL FIELD

[0002] The invention relates generally to devices and methods for the recovery of a target molecule from a sample. More specifically, the invention relates to devices and methods for the recovery of a target molecule utilizing a capture probe attached to a substrate.

## BACKGROUND OF THE INVENTION

[0003] Biological samples often include heterogeneous components, such as nucleic acids, proteins, and other cellular components. It can be desirable to recover those components for analysis. Components of a sample can be selectively recovered based upon size and charge density using an electric field in conjunction with a solid support matrix, such as an electrophoretic gel.

[0004] Recent developments in molecular biology provide great potential for detecting DNA mutations or alterations indicative of cancer and other diseases. For example, EXACT Sciences Corporation has found that mutations indicative of various stages of colorectal cancer can be detected in DNA found in stool samples. Detecting the presence of cancer from a stool sample is non-invasive, as opposed to a colonoscopy, and more specific to cancer than a fecal occult blood test (FOBT), which tests for the presence of blood that could indicate one of a number of different conditions.

**[0005]** Use of the polymerase chain reaction (PCR) has made the detection of nucleic acids more routine, but PCR is limited by the initial amount of target DNA present in a sample. A minimum initial amount of DNA must be present to amplify relevant amounts of the target. Target DNA in a sample in small amounts may not be amplified to any significant extent in PCR. For example, due to the stochastic nature of PCR, if a low-frequency DNA is not amplified in the first few PCR rounds, low frequency DNA might not be detectable over background. More specifically, if a mutant is present in approximately 1% of instances of a locus in a sample, then at least 100 copies of the locus must be used to amplify the single mutant copy within.

**[0006]** An additional problem encountered in preparing stool samples to detect human DNA is that it is difficult to extract sufficient quantities of human DNA from the stool. Human DNA in stool is typically present in low concentration, and is diluted by high concentrations of contaminating DNA (e.g., from bacteria). In addition, DNA in stool often is degraded or partially degraded, which can reduce the efficiency of extraction methods. Furthermore, stool samples routinely contain cell debris, enzymes, bacteria (and associated nucleic acids), and various other compounds that can interfere with traditional DNA extraction procedures and reduce DNA yield.

**[0007]** Recovery of target nucleic acids from stool and other heterogeneous tissue and body fluids using sequence-specific hybrid capture can be inefficient. Electrophoretically driven affinity capture using gel-based solid phases with immobilized sequence-specific capture probes is one approach that has been used to overcome these inefficiencies.

**[0008]** It would be desirable to process large volumes of crude DNA (including in the form of stool homogenate) to avoid a need for high concentrations of sequence-specific capture probes

to extract from a sample sufficient quantities of target DNA, to reduce the complexity of a crude DNA sample, and to improve the capture efficiency.

## SUMMARY OF THE INVENTION

**[0009]** The present invention includes devices and methods for recovering target molecules from stool or other heterogeneous biological samples using affinity capture driven by a motive force such as electricity, pressure, or a vacuum. The target molecules can then be used for further analysis. With a stool sample, target DNA can be used to detect whether a patient has a disease or condition, such as colorectal cancer.

**[0010]** Certain embodiments of the invention utilize electrophoresis and nucleic acid hybridization for recovering and purifying target DNA. The devices and methods can recover a target nucleic acid that is electrophoresed through a medium and that binds to nucleic acid or nucleic acid analog capture probes (e.g., peptide nucleic acid (PNA) probes). Such target nucleic acids include, for example, DNA and RNA molecules. Specifically encompassed by embodiments of the present invention is the recovery of target nucleic acids from heterogeneous biological samples containing DNA, such as stool samples or saliva samples. In other embodiments, proteins, peptides, and lipids that bind to nucleic acid or nucleic acid analog capture probes, can be recovered as the target molecule.

**[0011]** In one aspect, the invention relates to a device for recovering a target molecule from a biological sample. The device includes a first unit defining a chamber, and the chamber includes a first region for receiving a sample and a second region including at least one electrophoretic medium. The device includes a second unit extending from a first end to a second end, and the second unit is interfaceable with the first unit. A mesh structure is impregnated with an electrophoretic medium that includes capture probes copolymerized thereto and is located at the

second end of the second unit. The second region is located between the first region and the mesh structure when the first unit is interfaced with the second unit.

**[0012]** The aspects described above or below can have any or all of the following features. The device can be oriented such that the first unit and second unit, when interfaced, are in a vertical or horizontal orientation. The first unit can be a tube. The first unit can include a support at its end. The support can be at least one of a ridge, a solid support, or a mesh. The electrophoretic medium of the first unit and the electrophoretic medium of the second unit can include the same or different materials. The first unit can also include one or more additional electrophoretic media. The invention can also have a plurality of first units and a plurality of second units. The first unit can be capable of nesting within the second unit. The mesh structure can be attached to the second unit at the second end, and the mesh structure can include a non-conductive material. The electrophoretic medium of the second unit can include a plurality of identical or different capture probes. The capture probes can be substantially evenly dispersed within the electrophoretic medium of the second unit. A third unit can be interfaceable with the second unit, and the third unit can define a well. The second unit can be capable of nesting within the third unit.

**[0013]** In another aspect, the invention relates to a device that includes a housing defining a chamber. The chamber includes a first region for receiving a sample and a second region having at least one electrophoretic medium. The device also includes a mesh structure impregnated with an electrophoretic medium that includes copolymerized capture probes. The second region is located between the first region and the mesh structure, and the mesh structure is located at an end of the device.

**[0014]** The aspects described above or below can have any or all of the following features. A third unit defining a well can be interfaceable with the end of the device. The electrophoretic

material of the second region and the electrophoretic medium of the mesh structure can include the same material. The electrophoretic medium of the second region and the electrophoretic medium of the mesh structure can be a different material.

**[0015]** In another aspect, the invention includes a method for recovering a target molecule from a sample by application of a vacuum to a sample that contains a target molecule, in order to move the sample from a first area to a second area. An electric motive force then can be applied to the sample to move at least the target molecule in a first direction such that the target molecule interacts with a capture probe copolymerized to an electrophoretic medium. The aspects described above or below may also include a method of releasing the target molecule from the capture probe and subsequently applying a second electric motive force to move the target molecule in a second, different direction.

**[0016]** In another aspect, the invention relates to a device for processing biological samples. The device includes a first unit having a first well defined by a first set of walls and a first floor. The first floor includes a first plurality of openings and a first plurality of tubes, and each of the first plurality of tubes extends downwardly from the floor at one of the openings.

**[0017]** The aspects described above or below can have any or all of the following features. The first well and first plurality of tubes can be formed together as a unitary piece. The tubes can each have ridges at an end distal from the well. An electrophoretic gel at the distal end of each of the first plurality of tubes can be surrounded by the ridges. A mesh at the distal end of at least some of the tubes can also hold the gel in the tube. The mesh and tubes can be made of the same material and bonded together.

**[0018]** The first unit can interface with a second unit with a second well that is defined by a second set of walls and a second floor. The second floor includes openings and a second plurality of tubes extending downward from the floor, and each of the second plurality of tubes is

formed at an opening in the second floor. The second plurality of tubes can be of a size and shape so that the first plurality of tubes can nest therein. A first electrophoretic medium can block each of the second plurality of tubes. The first electrophoretic medium can include immobilized, sequence-specific capture probes for affinity binding to nucleic acids. The capture probes can hybridize with nucleic acids indicative of colorectal cancer that can be found in a stool sample. The outer diameter of each of the first plurality of tubes can be similar to the inner diameter of each of the second plurality of tubes, and each can be cylindrical in cross-section. The device can have a second electrophoretic medium suitable for blocking the passage of molecules, such as proteins, in the first plurality of tubes while allowing the passage of nucleic acids during electrophoresis. The second well and the second plurality of tubes can be formed as an integral unit.

**[0019]** In another embodiment, there can be a collection plate having a body and including a plurality of wells. The wells can have a cross-section configured so that the second plurality of tubes can nest in the wells. There can be 24(n) tubes, where n is a natural number. The first plurality of tubes can be at least 1 inch long each, and the first well can be at least 0.5 inches (1.27 cm) deep and at least 2 inches (5.08 cm) on each side. At the end of each of the first plurality of tubes there can be an electrophoretic medium including immobilized, sequence-specific capture probes for affinity binding to target nucleic acids. The capture probes can hybridize with nucleic acids indicative of colorectal cancer that can be found in a stool sample. There can also be a collection unit having a body and a plurality of wells configured to nest with the second plurality of tubes. The second unit can be substantially identical to the first unit and can include extender tubes between the tubes of the first unit and the tubes of the second unit. The combined first unit, second unit, and extender tubes can create a plurality of sample holding

conduits, the volume of the conduits can be selected to hold the sample plus any osmotic expansion due to electrophoresis.

**[0020]** In another aspect of the invention, a method for affinity capture of target nucleic acids from biological samples using any of the devices described above or below with any of the features described above or below, includes electrophoresing samples through the tubes so that target nucleic acids can bind to the capture probes.

**[0021]** The aspects described above or below can have any or all of the following features. The captured target nucleic acids may be eluted from the tubes. After elution, a collection plate with collection wells can be positioned under the tubes so that the eluted target nucleic acids are collected in the collection wells. A second electrophoretic medium without immobilized capture tubes can be introduced into the tubes prior to adding the biological samples, with the second electrophoretic medium being over the first medium and under the samples. The second electrophoretic medium can provide a barrier to prevent the sample from leaking through or around the electrophoretic medium with the immobilized capture probes. A screening assay can be performed on collected target nucleic acids to detect indicia of disease in the plurality of samples. The biological samples can be stool samples and the disease can be cancer or pre-cancer, including colorectal cancer. The screening assay can be selected from the group consisting of enumerated loss of heterozygosity (LOH), DNA integrity assay, mutation detection, expression assays, and fluorescence in situ hybridization (FISH). The sample unit can include a first unitary piece that includes the well and the tubes, as well as a second unit for holding the electrophoretic medium with the immobilized capture probes. The second unit can also include a second well with walls and a floor and a second plurality of tubes extending downwardly from the floor. The electrophoretic medium with the immobilized capture probes can extend across openings at lower ends of the second plurality of tubes. The second

electrophoretic medium without capture probes can be placed into the first plurality of tubes to provide a physical barrier over the electrophoretic medium with the immobilized capture probes. Electrophoresing can cause some non-target molecules to become trapped within the second electrophoretic medium without capture probes, and can further cause target nucleic acids to bind to capture probes in the electrophoretic medium with capture probes. The first unit can be physically separated from the second unit such that the second electrophoretic medium may be removed. A buffer solution can be added over the electrophoretic medium with capture probes and bound target nucleic acids and a reverse electrophoresis process can be performed. The electrophoretic medium with capture probes and bound target nucleic acids can be denatured in order to cause the target nucleic acids to separate from the capture probes. The electrophoresing can include placing the sample unit into a housing with a first set of electrodes near a lower end of the tubes and a second set of electrodes. The second set of electrodes can move downward from a leading position to a first operating position into the upper end of the tubes and can also facilitate the reverse electrophoresis process by moving to a second operating position.

**[0022]** In another aspect, the invention includes a method for affinity capture of target nucleic acids from biological samples with a device that includes an electrophoretic medium with immobilized capture probes for binding to target nucleic acids. The method includes adding a first electrophoretic medium into a first plurality of downwardly extending tubes and placing the first plurality of tubes into a second plurality of tubes that has a second electrophoretic medium with immobilized capture probes. In this method, the first medium is over the second medium. Different biological samples can be placed into different tubes of the first plurality of tubes so that the sample is on the first medium. A buffer can cover the samples and the samples can be electrophoresed downward through the tubes so that target nucleic acids in the samples can bind to the capture probes.

**[0023]** The aspects described above or below can have any or all of the following features. The first plurality of tubes can be removed from the second plurality of tubes after the electrophoresing. The first plurality of tubes can be configured so that the first medium remains held within the first plurality of tubes when they are separated from the second plurality of tubes.

**[0024]** Another aspect of the invention is a method for capturing molecules indicative of colorectal cancer. This method includes a first electrophoretic medium provided across a number of tubes in a first plurality of tubes. The first electrophoretic medium includes immobilized capture probes for binding to nucleic acids that are indicative of colorectal cancer and that may be found in a stool sample. A second electrophoretic medium can be placed over the first electrophoretic medium in order to block the passage of proteins during electrophoresis. A plurality of samples may be added into the tubes over the second electrophoretic medium. A buffer solution can be added over the samples and the stool samples can be electrophoresed so that target nucleic acids can bind to the capture probes in the first electrophoretic medium while non-target molecules can be trapped in the second electrophoretic medium.

**[0025]** The aspects described above or below can have any or all of the following features. The first plurality of tubes can be part of one unit, and there can be a second unit that has a second plurality of tubes that nest within the first plurality of tubes. The second electrophoretic medium, the samples, and the buffer solution can be added into the second plurality of tubes. The second plurality of tubes with the second electrophoretic medium, the samples that remain after electrophoresis, and the buffer solution from the second plurality of tubes, can be removed. Reverse electrophoresis can be performed on the first electrophoretic medium with the captured target nucleic acids. The first electrophoretic medium with the captured target nucleic acids can be denatured to separate the target nucleic acids.

**[0026]** Another aspect of the invention relates to an electrophoretic device for capturing target nucleic acids from biological samples. The electrophoretic device can include a chamber for receiving a sample and a capture chamber having an electrophoretic medium with immobilized, sequence-specific capture probes for affinity capture of target nucleic acids from the samples. The device can have electrodes for moving the target molecules along a first direction to bind with the capture probes when current is applied. Additional electrodes can be configured to cause target nucleic acid to migrate in a second direction perpendicular to the first direction. In certain embodiments, the invention can have a port for receiving a denaturing solution into the capture chamber.

**[0027]** Another aspect of the invention includes a method for capturing target nucleic acids from biological samples where a sample is added into a chamber and electrophoresed so that target nucleic acids within the sample pass through a capture chamber having an electrophoretic medium with immobilized, sequence-specific capture probes for affinity capture of target nucleic acids from the samples. The electrophoresing can include using electrodes to cause the target nucleic acids to move along a first direction to bind with the capture probes, allowing non-target DNA and other biological components to pass through, and subsequently denaturing the target nucleic acids from the capture probes in the capture chamber to recover them. Electric potential can be applied to cause the denatured target nucleic acid to migrate to a port for removal along a second direction perpendicular to the first direction.

**[0028]** Another aspect of the invention relates to an electrophoretic unit. The unit can have a housing for enclosing a first and a second set of sample tubes having first and second different heights. There can be a set of lower electrodes near lower ends of the first or second set of sample tubes when the first or second set is in the housing. There can also be a set of upper electrodes vertically movable between an uppermost loading position, in which the tubes can be

moved into or out of the housing, a first operating position below the loading position, in which the upper electrodes are in the upper end of the first set of tubes. There can also be a second operating position below the first operating position in which the upper electrodes are brought down into the upper ends of the second set of tubes. In various embodiments of the foregoing aspect, there can be a switch for determining the direction of polarity automatically in response to whether the upper electrodes are in the first or second operating position.

**[0029]** These and other objects, along with advantages and features of the present invention herein disclosed, will become apparent through reference to the following description, the accompanying drawings, and the claims. Furthermore, it is to be understood that the features of various embodiments described herein are not mutually exclusive and can exist in various combinations and permutations.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0030]** In the drawings, like reference characters generally refer to the same parts throughout different views. Also, the drawings are not necessarily to scale, emphasis instead generally being placed upon illustrating principles of the invention. In the following descriptions, various embodiments of the present invention are described with reference to the following drawings.

**[0031]** FIG. 1 is a schematic side view of an embodiment of a purification module constructed in accordance with an embodiment of the present invention.

**[0032]** FIG. 2 is a schematic perspective view of a volume extender plate constructed in accordance with an embodiment of the present invention.

**[0033]** FIG. 3 is a schematic perspective view of a capture plate constructed in accordance with an embodiment of the present invention.

**[0034]** FIG. 4 is a schematic perspective view of a collection plate constructed in accordance with an embodiment of the present invention.

**[0035]** FIG. 5 is a schematic cross-sectional view of the volume extender plate of FIG. 2 attached to the capture plate of FIG. 3.

**[0036]** FIG. 5A is a schematic part cross-sectional, part side view of tubes of a volume extender plate similar to that of FIG. 2 and a capture plate of FIG. 3.

**[0037]** FIG. 5B is a schematic side view of an electrophoresis unit for providing a voltage to a group of samples in a system such as one shown in FIG. 5 or FIG. 6.

**[0038]** FIG. 6 is a schematic cross-sectional view of the capture plate of FIG. 3 attached to the collection plate of FIG. 4.

**[0039]** FIGS. 7(A) – 7(C), respectively, are schematic top and cross-sectional side views of the collection plate of FIG. 4, and FIG. 7(D) is a schematic cross-sectional detailed view of FIG 7(B).

**[0040]** FIGS. 8(A) – 8(C), respectively, are schematic top and cross-sectional views of the capture plate of FIG. 3.

**[0041]** FIGS. 9(A) – 9(C), respectively, are top and cross-sectional views of the volume extender plate of FIG. 2.

**[0042]** FIGS. 10(A) and 10(B), respectively, are schematic end and cross-sectional views of a sample tube of the volume extender plate of FIG. 2, and FIG. 10(C) is a schematic cross-sectional detailed view of FIG 10(B).

**[0043]** FIG. 11 is a schematic cross-sectional view of another embodiment of an affinity capture purification device constructed in accordance with an embodiment of the present invention.

**[0044]** FIG. 12 is a schematic perspective view of the affinity capture purification device of FIG. 11.

**[0045]** FIG. 13 is a schematic cross-sectional view of an affinity capture purification device constructed in accordance with a further embodiment of the present invention.

**[0046]** FIG. 14 is a schematic cross-sectional view of an affinity capture purification device constructed in accordance with a still further embodiment of the present invention.

**[0047]** FIG. 15 is a schematic cross-sectional exploded view of individual tubes used for the electrophoresis of samples.

**[0048]** FIG. 16 is a schematic perspective view of a unit for receiving samples, including two volume extender plates of the type shown in FIG. 2 and extending tubes therebetween.

## DESCRIPTION

**[0049]** Systems, devices, and methods are provided for selectively separating and purifying specific target molecules from biological samples using a motive force such as electricity, pressure, or a vacuum. In the case of electrophoresis, target molecules can include DNA, RNA, proteins, and lipids. While the specification highlights target molecules that are nucleic acids, it should be understood that any molecule that binds to a capture probe of the device is contemplated. One or more electrophoretic media can be used. If more than one medium is used, a biological sample, such as a homogenized stool sample, can be placed in a device that has a first electrophoretic medium and a second electrophoretic medium. The first electrophoretic medium impedes the passage of non-target molecules (e.g., proteins and other non-nucleic acids) and thus has a filtering effect. The second electrophoretic medium has immobilized, sequence-specific capture probes that bind to target molecules such as nucleic acids. The biological sample is electrophoresed through the first electrophoretic medium and the second electrophoretic medium such that some non-target molecules are impeded in the first

electrophoretic medium and target molecules are captured by immobilized capture probes in the second electrophoretic medium.

**[0050]** Heterogeneous biological samples for use with the invention can be any sample containing nucleic acids, proteins, and/or lipids and can be obtained from any source. Specifically encompassed by the present invention are heterogeneous samples from biological sources containing DNA, such as stool samples, saliva samples, blood samples, including whole blood, blood plasma, and blood serum, and any samples containing exfoliated cells and/or cellular debris. The heterogeneous biological sample is treated to render target nucleic acids contained in the sample available for binding. For example, a cell lysate can be prepared, and the crude cell lysate (e.g., containing the target nucleic acids as well as other cellular components) can be analyzed. Alternatively, a target nucleic acid can be partially isolated (rendering the target substantially free from other cellular components) prior to analysis.

**[0051]** A target nucleic acid is any nucleic acid of interest that can form a binding complex with a capture probe, e.g., a target nucleic acid can have a sequence that is complementary to a sequence of the capture probe and thus hybridize to it. Nucleic acids (as both targets and capture probes) include DNA, RNA, modified nucleic acids, and nucleic acid analogs such as peptide nucleic acid (PNA) and morpholino nucleic acids. Both single stranded and double stranded nucleic acids are embraced by embodiments of the invention.

**[0052]** A capture probe is any nucleic acid that can form a specific binding complex with a target molecule, such as a nucleic acid, and can be immobilized within a suitable electrophoretic medium. When a capture probe forms a stable specific binding complex with a target, the mobility of the target (i.e., the speed at which the target migrates through the medium) is lowered. In one embodiment, the entire nucleotide sequence of the target is complementary to the capture probe. In another embodiment, only a portion of the target nucleotide sequence is

complementary to the capture probe. For example, the target may be a sequence with a single polymorphic site comprising one or more nucleotides that are mismatched with the capture probe.

**[0053]** Probes can be immobilized by direct attachment to polymeric components of the medium with covalent bonds or non-covalent bonds, although covalent attachment is typical. Immobilized nucleic acid probes capture complementary sequences in heterogeneous biological samples for purification and recovery. An electrophoretic medium that allows for direct recovery of captured target nucleic acids for downstream applications (e.g., polymerase chain reaction, mutational analysis) improves probe density, capture efficiency, and separation capability.

**[0054]** A first electrophoretic medium can be any medium suitable for electrophoresis. The first electrophoretic medium may help to provide a water tight seal when the invention is assembled. In addition, the first electrophoretic medium may be selected and altered to impede the passage of non-target molecules. In one embodiment, the first electrophoretic medium can be an agarose, acrylamide, or polyacrylamide gel medium, but other electrophoretic media can be used. A pore size of the first electrophoretic medium may be adjusted to accommodate the desired target nucleic acids and to exclude or impede the passage of non-target molecules. In one embodiment, the first electrophoretic medium has immobilized, sequence-specific capture probes for binding non-target molecules. In another embodiment, the first electrophoretic medium has two or more types of capture probes with a broad spectrum of specificity for capturing non-target molecules.

**[0055]** A second electrophoretic medium can be any medium suitable for electrophoresis in which capture probes can be immobilized in the medium, and typically is a medium in which capture probes are dispersed throughout the medium by being copolymerized within the medium.

The second electrophoretic medium can include agarose, acrylamide, or polyacrylamide gel, but other electrophoretic media can be used. Target nucleic acid, such as DNA, can be recovered from the second electrophoretic medium for use in assays, including, for example, PCR. In one embodiment, the second electrophoretic medium can be dissolved by disrupting covalent crosslinks to render the medium soluble. In this case, the electrophoretic medium is designed to include crosslinking moieties that are reversible by addition of specific chemicals. For example, addition of NaOH will hydrolyze diester crosslinks, as supplied, for example, by ethylene glycol diacrylate (EGDA) crosslinkers. By dissolving electrophoretic media after capture and washing, captured nucleic acid, such as DNA, can be recovered quantitatively. Alternatively, heat or alkaline conditions can be used to disrupt base pair-pairing interactions between the capture probe and target nucleic acid, which allows recovery of target nucleic acid, such as DNA. Additionally, the elution buffer can be separated from the electrophoretic medium by centrifugation. In another embodiment, target nucleic acid, such as DNA, may be driven from the electrophoretic medium by applying a voltage, or a voltage in combination with a previously described method of disrupting base-pairing interactions, sufficient to drive the target nucleic acid from the electrophoretic medium.

**[0056]** In one embodiment, capture probes are uniformly immobilized throughout the second electrophoretic medium. In another embodiment, more than one type of capture probe is immobilized throughout the second electrophoretic medium. In a further embodiment, two or more regions of similar or different immobilized capture probes, or combinations of probes, are positioned in the second electrophoretic medium such that a biological sample migrates sequentially through multiple regions of immobilized capture probes in the second electrophoretic medium. Multiple capture regions can be useful to capture different nucleic acid

species. During electrophoresis, a biological sample is electrophoresed through each region of the second electrophoretic medium, where complementary target nucleic acids are captured.

**[0057]** A general description of an affinity purification device for recovering a target nucleic acid, such as DNA, from a stool sample, is provided with reference to FIG. 1. Target DNA is moved through an electrophoretic module 10 by applying an electric potential from top to bottom. Electrodes (not shown) are provided above and below the electrophoretic module 10 to drive DNA through the module. An entire volume of a stool sample (preferably in a processed and homogenate form) is introduced at a top layer 12 of the electrophoretic module 10. The electrophoretic module 10 has different regions to effect differential separation in a single pass and allow for continuous processing of crude DNA.

**[0058]** An upper layer 14 is a filtration layer that can be constructed to remove particulates and aggregates from a crude heterogeneous sample. A secondary layer 16 can have a size-exclusion material that prevents the passage of large molecular weight fractions, and typically includes agarose or acrylamide gel. A third layer 18 has a sequence-specific capture zone. The composition of the secondary layer 16 can be selected to remove some of the high molecular weight DNA before a sample reaches the third layer 18. In certain embodiments, the third layer has oligonucleotides covalently attached to an acrylamide gel or acrylamide copolymer. The electrophoretic module can have any combination of the layers, but must at least include the third layer (i.e., the upper layer and/or the secondary layer may be excluded). To process large volume samples or to capture greater quantities of target DNA, the depth of the third layer can be increased, or the diameter of the electrophoretic module can be increased. The binding capacity of the electrophoretic module, and particularly of the third layer, affects the amount of target DNA recovered.

**[0059]** FIGS. 2-10 show an assembly for creating multiple electrophoresis columns including a sample receiving unit, referred to as a volume extender plate 20 (e.g., FIGS. 2, 5, 5A, 9A- 9C, and 10A- 10C), a capture plate 30 (e.g., FIGS. 3, 5, 6, and 8A- 8C), and a collection plate 40 (e.g., FIGS. 4, 6, and 7A- 7D). Referring to FIG. 2, the volume extender plate 20 has a generally rectangular buffer well 28 defined by four upstanding walls 23 and a floor 25. The floor 25 has openings 26, typically (but not necessarily) arranged in a uniform and compact two-dimensional manner. Extending downward from the floor 25 at the openings 26 are sample tubes 24, each defining a fluid-containing volume. The buffer well 28 is thus in fluid communication with all of the sample tubes 24 at the same time. The buffer well 28 provides a relatively large volume of electrophoresis buffer in contact with the samples placed in the sample tubes 24. In an alternative embodiment, the buffer can be segregated to the individual sample tubes by inserting interior walls that divide the buffer well.

**[0060]** As shown in the cross-sectional view in FIG. 5, lower ends of the sample tubes 24 can have a threading or some other roughened or non-smooth surface 33 that increases friction in order to hold an electrophoretic medium or other material in the same sample tubes 24. Additionally or alternatively, a supporting mesh floor 41 as shown in FIG. 5A, can be included for holding the electrophoretic medium or other material that serves as a plug (discussed below). Additional views of the volume extender plate 20 are shown in FIGS. 9A- 9C and 10A- 10C.

**[0061]** Referring to FIG. 3, the capture plate 30 has a generally rectangular buffer well 39 defined by four upstanding walls 32 and a floor 36. The floor 36 has openings 34, and, at the openings 34, capture tubes 38 extend downward from the floor 36 and are arranged in the same manner as the sample tubes 24 (FIG. 2). A membrane 35 is provided across, and typically is bonded to the bottom of, each of the capture tubes 38. The membrane 35 is a mesh support material, such as polyester or another suitable inert polymer mesh (which may be non-

conductive), impregnated with an electrophoretic medium, such as an agarose or polyacrylamide gel, having copolymerized capture probes that capture target nucleic acids of interest that contact the membrane 35. There are typically separate pieces of the membrane 35 at the end of each of the capture tubes 38. However, one membrane sheet can cover the bottom of all of the capture tubes. Additional views of the capture plate 30 are shown in FIGS. 5, 6, and 8A- 8C.

**[0062]** The membranes can be prepared in batch mode between glass plates using a medical grade polyester sheet as a physical support for the electrophoretic medium. To the extent a polyacrylamide gel is the electrophoretic medium, a polyacrylamide matrix is prepared by copolymerization of acrylamide monomer, an Acrydite®-functionalized oligonucleotide capture probe (i.e., an oligonucleotide with an acrylamide group added to its 5' end) (Mosaic Technologies, Boston, MA), and a crosslinker such as methylene bisacrylamide. The sequences of the capture probe are defined by standard base-pairing rules in order to bind a target gene of interest. The length of the probes and concentration can be manipulated to optimize DNA recovery. After polymerization is complete, the glass plates can be removed and the membrane may be cut into an appropriate shape in order to be welded to the bottom of the capture plate wells. In one embodiment, welding the membrane to the capture plate well can be achieved by locally heating the edges of the membrane and capture wells in order to melt the membrane to the well. Alternatively, the electrophoretic medium can be directly polymerized. For example, instead of preparing the membrane by polymerizing the electrophoretic medium in the presence of a support mesh, the electrophoretic medium can be poured directly onto a porous support on the bottom of a tube. Polymerization is then initiated with ammonium persulfate and tetramethylenediamine (as described below) or by photopolymerization (with standard photoinitiators). In certain embodiments, relatively thicker electrophoretic medium layers can be prepared, eliminating the steps of preparing, drying, and bonding membranes.

**[0063]** Referring to FIG. 5, the volume extender plate 20 and the capture plate 30 are shown nested together. Once nested together, a bolus of an electrophoretic medium, such as agarose gel, is introduced through the opening 26 of the volume extender plate 20 into the bottom of the sample tubes 24 so that it is over, and preferably directly on top of, the membrane 35 at the end of the capture tubes 38 of the capture plate 30. The agarose gel or other medium forms a plug 37 that serves as a filter above the membrane 35 for particulates and larger molecules, and helps to seal leaks in the membrane 35 or to fill in gaps between the nested sample tubes 24 and the capture tubes 38. One plug 37 is shown, but typically there would be one over every piece of the membrane. Although this embodiment (as well as some of the other embodiments described herein) is shown in a vertical orientation, other embodiments can generally have these configurations but be oriented horizontally. When oriented horizontally, leaks that might drain the sample through the membrane before it could be electrophoresed are less of a problem.

**[0064]** The electrophoretic medium, such as the agarose gel, can be introduced into the sample tubes 24 from the openings 26 and fall to the bottom of the sample tubes 24. Alternatively, the electrophoretic medium can be placed in the capture plate over the membrane so that when the sample tubes are pushed down over the medium, the medium moves up into lower portions of the sample tubes.

**[0065]** Referring now to FIG. 5A, which is an assembly similar to the embodiment shown in FIG. 5 except for the inclusion of a supporting mesh 41 at the end of the sample tube 24, one sample tube 24 is shown nested in one capture tube 38 (only one of the plurality of these tubes are shown). A sample 45 (such as a stool homogenate in liquid form) is introduced into the sample tube 24 and on top of the plug 37, shown over the supporting mesh 41. The same sample can be introduced into a number of different sample tubes while another sample is also placed in a number of sample tubes, or each sample tube can be uniquely associated with a different

sample. Control samples can also be provided in some of the sample tubes. For example in a 48-tube unit, four samples for each of 10 patients, 2 samples for each of 20 patients, or one sample for each of 40 patients can be added into sample tubes, leaving eight sample tubes for control samples.

**[0066]** A buffer solution 47 is introduced into the buffer well 28, causing the buffer solution to fill some or all of the remainder of each sample tube 24 over the sample 45. The samples are put into the sample tubes such that the upper level of the samples within the sample tubes are below the floor of the volume extender plate. This prevents contamination across sample tubes that can contain samples 45 from different individuals when the buffer is added into the buffer well. In this way, buffer can be added at one time to all the samples. A lowermost part of the capture tube 38 is immersed in a buffer in a lower buffer chamber 49.

**[0067]** An upper electrode 48a is introduced into the buffer well 28 and sample tube 24 and a lower electrode 48b is introduced into the lower buffer chamber 49. Then, a current is applied from a voltage source through the vertical distance of the sample tubes 24 (only one sample tube 24 is shown for clarity) and the capture tubes 38 (e.g., at about 1 to about 200 volts for a desired amount of time) to produce multiple parallel electrophoretic columns for the samples. Under the influence of the voltage and resulting electric fields, charged DNA migrates from the stool sample 45, through the plug 37, and across the membrane 35 where target nucleic acids bond with capture probes. The upper electrode 48a is typically vertically elongated to extend into the sample tube 24 above the sample 45. The lower electrode 48b is typically in the buffer contained within the lower buffer chamber 49, but placed at a vertical position that is above the level of the membrane 35.

**[0068]** FIG. 5B illustrates an electrophoresis unit 120 for providing a voltage to samples in a unit such as that shown in FIGS. 5 and 5A. An upper plate 100 has a dielectric support and a

number of electrodes and is movable between an upper loading and unloading position (as shown in solid line) and one or more lower operating positions (one of which is shown in phantom). The upper plate 100 has a horizontal guide plate 102 with openings that allow the upper plate 100 to move vertically along guide rails 104, 106 to control its vertical position. The upper plate 100 can be moved manually or with a motorized, automated system. On its underside, the upper plate 100 has a plurality of downward extending electrodes 108 (four of which are shown) that are mounted to a dielectric support 118 and are electrically coupled to a voltage source through a fixture 110 and conductors 112.

**[0069]** Another fitting 114 and conductor 116 provide electrical connection to electrodes (not shown) located in the lower buffer chamber 49, in which the capture plate 30 rests. As indicated above and in FIG. 5A, these electrodes typically are at a vertical level above the membrane with capture probes.

**[0070]** The sample tubes 24 of the volume extender plate 20 and the capture tubes 38 of the capture plate 30 are shown in dashed line. As indicated in FIG. 5B, when the upper plate 100 is in its upper position, the electrodes 108 are spaced from the sample tubes 24, and when the upper plate 100 is moved to a lower operating position, the electrodes 108 extend into the sample tubes 24. When the upper plate 100 is in the lower operating position, the voltage source can be energized to provide the voltage for electrophoresis. In FIG. 5B, the electrodes 108 are shown with pointed ends, but they can have blunt ends and can be made longer than they appear in FIG. 5B, e.g., up to 5 inches (12.7 cm) with 4 inches (10.2 cm) extending into the sample tubes.

**[0071]** The electrophoresis unit 120 is enclosed by a housing that has two parts, a cover 122 and a base 124, that separate along a horizontal line 126. In use, the cover 122 is removed, and the assembled volume extender plate 20 and capture plate 30 are placed into the base 124. The sample and buffer can be introduced into the assembly before or after the volume extender plate

20 and the capture plate 30 are in the unit 120. The cover 122 is then placed over the base 124 and the upper plate 100 is brought down, typically manually with a mechanical slide that extends outside the housing, so that the electrodes 108 pass through the buffer well 28 and extend into the sample tubes 24.

**[0072]** Electrophoresis can be performed with a continuous voltage or with a pulsed field gel electrophoresis (PFGE) technique. The PFGE process includes alternating the direction of electrical current in a pulsed manner across the gel to help separate the molecules. PFGE can facilitate the separation of larger DNA fragments and can be performed during the initial electrophoresis prior to removal of the volume extender plate.

**[0073]** The upper plate 100 of the electrophoresis unit 120 can move over a range of positions, but has at least three positions: a top position, in which the unit is open to add or remove a volume extender plate with or without samples; a first operating position, in which the electrodes are inserted into sample tubes in a volume extender plate to provide electrophoresis in a first direction; and a second, lowermost operating position, in which the electrodes extend even further downward into the capture plate and electrophoresis is provided in the capture plate, without the volume extender plate, and optionally, electrophoresis is provided in a second, opposite direction. The electrophoresis unit 120 can have a detector or switch, such as a reed switch, that controls the direction of electrophoresis based on the operating position of the upper plate 100.

**[0074]** After electrophoresis is performed in the first operating position, the remaining buffer and sample are poured out of the sample tubes 24, and the volume extender plate 20 is physically separated from the capture plate 30. Ridges 33 at the bottom of the sample tubes 24 provide increased friction to help keep the plug 37 in place so that it is extracted along with the volume extender plate 20 as it is removed from the capture plate 30. The volume extender plate 20 can

be cleaned and sterilized for further use or discarded. The capture plate 30 is rinsed, and the capture tubes 38 are again placed in the electrophoretic unit 120 in a chamber containing fresh buffer solution. Fresh buffer solution can also be added to the buffer well 39 shown in FIG. 5 so that it fills, at least in part, the capture tubes 38 with a sufficient volume to at least cover each membrane 35.

**[0075]** If the geometry of the sample tubes 24 and the type of electrophoretic medium that is used to form the plug 37 have insufficient stability such that it is impossible to remove the plug 37 intact, i.e., if pieces or portions of the plug are typically left behind, an additional supporting mesh 41 can be added to the bottom of the tubes 24 to better ensure that the plugs 37 are removed with sample tubes 24 (FIG. 5A). The supporting mesh can be made from any desirable material, but is preferably chosen so that it is easy to bond the supporting mesh to the sample tubes. For this purpose, it can be desirable to have the supporting mesh and the sample tubes made of the same material to allow the supporting mesh to be ultrasonically bonded to sample tubes, rather than heat-bonding. In this case, both structures could be made of polypropylene. The supporting mesh can be bonded in small circles to the bottom of the sample tubes, or a sheet of the supporting mesh can be bonded to the bottom of all of the sample tubes at the same time, and, then, undesired portions can be removed, such as with laser cutting.

**[0076]** The size of the pores through the supporting mesh, and the percent porosity, can vary as desired but should preferably be small enough to provide a good ability to hold the electrophoretic medium in place, and but not too small to impede the flow of the electric field and/or the sample through the device. It has been found that one desirable pore size is one in which the aggregate opening size of the pores is about 50% (+/- 10%) of the total area of the supporting mesh. In other words, the total porosity of the mesh is about 50% (+/- 10%).

**[0077]** After the volume extender plate 20 is removed and the capture plate 30 is returned to the electrophoresis unit 120, the upper plate 100 of the electrophoresis unit 120 is placed in the lowermost operating position so that the electrodes 108 are placed into the capture tubes 38 from above, while other electrodes are already in the chamber in the base 124 under the membrane 35. The polarity of the electrodes is reversed from the prior electrophoresis process to drive non-target molecules (for example, those that are physically trapped on the upper surface of the membrane) out of the membrane 35. Following this reverse electrophoresis process, the capture tubes 38 are emptied of any material except the membrane 35, its capture probes, and any bound target molecules, and the capture plate 30 is again rinsed.

**[0078]** Electrophoresis can be run in at least two different configurations. In one embodiment, after the samples are loaded into the individual sample tubes, electrophoretic buffer is loaded into each of the sample tubes as well as into the buffer well, and a single electrode is inserted into the buffer well (as well as an additional electrode into a common lower buffer chamber, below the capture plate) to apply equal electric potential to each of the sample tubes. In another embodiment, after samples have been loaded into the individual sample tubes, electrophoretic buffer is added without completely filling the sample tubes, and an array of electrodes that matches the array of sample tubes is lowered into each of the tubes in order to apply an electric potential to the samples.

**[0079]** The captured DNA can then be removed or separated from the membrane 35 for further analysis. Referring to FIGS. 4, 6, and 7A – 7D, the capture tubes 38 of the capture plate 30 are placed into collection receptacles 44 in a collection plate 40. The collection plate 40 has a body 42 with the collection receptacles 44 positioned to correspond to the location of the capture tubes 38.

**[0080]** One method for recovering the target molecules includes adding a solution to dissolve the electrophoretic medium in the membranes 35. If the electrophoretic medium is dissolved, the assembly can be placed in a centrifuge to cause the electrophoretic medium, capture probes, and captured nucleic acids to be ejected from the membranes 35 into the collection receptacles 44. The capture plate 30 can be removed and discarded.

**[0081]** Samples in the collection receptacles 44 can be pipetted into other containers for further processing, or the collection plate 40 can be covered so that each collection receptacle 44 is closed, thereby allowing the samples to be stored without evaporation. The samples can be amplified (e.g., with PCR) and analyzed, e.g., for the presence of mutated DNA that is indicative of colorectal cancer. In this embodiment, the components of the electrophoretic medium, and the additives used to dissolve the electrophoretic medium, are chosen to be compatible with a PCR reaction such that they do not inhibit the reaction.

**[0082]** Alternatively, for some tests, it may be possible to detect the presence or absence of a captured target while it is still captured on the membrane, such as with optical detection methods. This detection can be accomplished using light sources and detectors, such as systems that include a light source for providing light at a desired wavelength, and a detector for detecting fluorescence. In such a case, target molecules could be first treated to have a fluorescent label or could be further bound to a signal probe that is detectably labeled and specifically binds to the target molecule.

**[0083]** Alternatively, the target molecules can be eluted from the capture probes without dissolving the medium. For example, the target molecules can be eluted using one or more of heat, addition of buffer to disrupt the capture complex, or high voltage electrophoresis. To the extent electrophoresis is used to drive released target molecules from the electrophoretic medium, a dialysis membrane can be configured below the electrophoretic medium while

electrodes are placed above and below the assembly. Electrophoresis drives the target molecules from the electrophoretic medium to the dialysis membrane, which can then be rinsed to collect the target molecules.

**[0084]** Embodiments of the present invention include 24, 48, or 96 (or more generically, 24(n), where n is a natural number) sample tubes, capture tubes, and collection receptacles in each device, to reduce the labor involved in running the process and preferably to mate with other existing equipment. These numbers are exemplary, and others could be used.

**[0085]** Referring to FIGS. 5 and 5A, the buffer well 28 of the volume extender plate 20 and the buffer well 39 of the capture plate 30 are integral with the plates. The plates can have multiple pieces, including tubes that are press fit into the respective buffer wells. More preferably, the volume extender plate is made of a unitary molded piece, monolithic in composition, except for the mesh (if used) for holding the plug. The capture plate also can be a unitary molded piece, monolithic in composition, except for the membrane (including the electrophoretic medium therein). The collection plate also can be a unitary molded piece monolithic in composition. The unitary pieces that make up the volume extender plate, the capture plate (except the membrane), and the collection plate, can be machined or are preferably injection molded from a suitable plastic or resin, such as polypropylene.

**[0086]** The membrane can be bonded to each of the capture tubes as circular (or other shaped) pieces. Alternatively, one piece of the membrane can be bonded to capture tubes, and if needed, portions of the membrane that do not cover a capture tube can be removed.

**[0087]** In one embodiment, the volume extender plate and capture plate are each on the order of inches in size. The sample tubes are typically at least 0.5 inches (1.8 cm) long and preferably up to 3 inches (7.6 cm) long, although somewhat shorter or somewhat longer tubes can be used. The sample tubes are preferably generally cylindrical and tapered with about 0.375 inch (1 cm)

outer diameter at the uppermost part. The capture tubes are also generally cylindrical and have about a 0.375 inch (.95 cm) inner diameter and a length of about 0.6 inches (1.52 cm). The buffer well of the volume extender plate is about 3 inches by about 5 inches (7.6 cm by 12.7 cm) and about 1 inch (2.5 cm) deep, but the size could vary although it would typically be at least 2 inches (5 cm) along the sides, and at least 0.5 inches (1.3 cm) deep, for a volume of at least 2 cubic inches (11.9 cubic cm). In one exemplary embodiment, the stool samples have a volume on the order of about 2 to about 6 ml each, with about 1 to about 3 ml of buffer for each sample.

**[0088]** As shown in FIG. 9A, the sample tubes 24 are preferably in a regular array such that the midpoint of one of the cylindrical tubes in one row is at the midpoint between two tubes in a next row. This configuration is useful to make a compact array, but other shapes and configurations of tubes can be used.

**[0089]** While the buffer wells 28, 39 are shown as rectangular, other shapes could be used, including cylindrical, in which case the term "walls" would encompass a continuous annular wall.

**[0090]** FIGS. 11-12 show another embodiment of the invention. An affinity capture purification device 50 has a tubular casing 52, typically made from a polymer. A biological sample is placed into an input well 54. A first filter barrier 56 prevents large particles from passing through to a buffer container 64. The sample is then drawn with a vacuum port 58 into the buffer container 64. A buffer input port 60 allows fresh buffer solution to be introduced into the buffer container 64. Electrodes 62 at opposite ends of the affinity capture purification device 50 provide an electric field that causes the sample to migrate through a second filter barrier 66 and into an electrophoretic medium 68 containing enteric capture probes. Enteric nucleic acids are captured in the electrophoretic medium 68 and the remaining sample migrates to an electrophoretic medium 70 that does not have capture probes. The sample further migrates to an

electrophoretic medium 72 containing target capture probes (e.g., immobilized within the electrophoretic medium 72), where target nucleic acids are captured in electrophoretic medium 72, while the remaining sample migrates to an electrophoretic medium 70 without probes.

**[0091]** Captured target nucleic acids are recovered by disassociating the captured target nucleic acids from the electrophoretic medium 72 and applying an electric field using the electrodes 86. A denaturing solution from a buffer chamber 88 flows through a porous panel 76 and disassociates the captured target nucleic acids. Target nucleic acids are captured in an elution receptacle 90 and collected from a sample removal port 74.

**[0092]** Another embodiment is shown in FIG. 13 that is similar to the embodiment shown in FIGS. 11 and 12, except for the addition of structures adjacent to the electrophoretic medium containing enteric capture probes. In this embodiment, captured enteric nucleic acids are recovered by disassociating the captured enteric nucleic acids from the electrophoretic medium 68 containing enteric capture probes and applying an electric field using electrodes 92. Denaturing solution from a buffer chamber 88 flows through a porous panel 76 and disassociates the captured enteric nucleic acids. Enteric nucleic acids are captured in an elution receptacle 90 and collected from a sample removal port 74.

**[0093]** Another embodiment is shown in FIG. 14 that is similar to the embodiment shown in FIGS. 11-12, except that it also has multiple capture regions 78, 80, 82, 84, each of which can have different capture probes. Captured nucleic acids are recovered by disassociating them from capture regions 78, 80, 82, 84 and applying an electric field using electrodes 92 (only one is labeled for clarity). Denaturing solution from buffer chambers 88 (only one is labeled for clarity) adjacent to each of the capture regions 78, 80, 82, 84 flows through each chamber's 88 porous panel 76 (only one is labeled for clarity) and disassociates captured nucleic acids. Nucleic acids are captured in elution receptacles 90 (only one is labeled for clarity) adjacent to

each of the capture regions 78, 80, 82, 84 and collected from each receptacle's 90 sample removal ports 74 (only one is labeled for clarity).

**[0094]** Devices and methods in these embodiments of the invention enable the processing of large volumes of a heterogeneous sample to recover large amounts of target DNA using a relatively small volume of electrophoretic medium. For example, if the concentration of probe is $20 \times 10^{-6}$ M, and the dimensions of the electrophoretic medium are 1 cm in diameter by $100 \times 10^{-6}$ m thick, the amount of available capture probe is about $9 \times 10^{13}$ molecules (the number of capture probe molecules is calculated according to the formula C x V x Av; where C = concentration of the capture probe, V = volume of the electrophoretic medium, and Av = Avegadro's number). If the hybridization efficiency is only 0.1%, it is theoretically possible to recover approximately $10^{10}$ target molecules of DNA before saturating the capture layer. If the target DNA were present at a concentration of 100 molecules/μl of crude mixture, up to $10^8$ μl (100 L) of crude mixture could theoretically be purified before the gel medium was saturated with target sequence.

**[0095]** Additionally, devices and methods in these embodiments of the invention provide for processing large volumes of heterogeneous sample using electrophoretically-driven affinity capture and removing non-target molecules prior to a capture in order to improve capture efficiency over other systems. The invention further provides the advantage of requiring lower concentrations of sequence-specific capture probe to extract sufficient quantities of target molecules from a heterogeneous biological sample. Applications of the present invention include automated sample recovery, high stringency DNA separations (e.g., mutant enrichment), and multiplexed separation of target sequences.

**[0096]** The system of FIGS. 2-10 has been shown with multiple tubes extending from a single well. In another embodiment and referring to FIG. 15, portions of the invention can also

be provided by using individual sample tubes and collection tubes, with processing that can be performed in whole or in part in an in-line manner. In this example, a capture tube 140 is provided with a mesh support membrane 142 impregnated with an electrophoretic medium containing copolymerized capture probes (as described in other embodiments) across capture tube 140, preferably at the lowermost end of the tube.

**[0097]** A sample tube 144 fits within the capture tube 140. An electrophoretic plug 146 as described above, typically agarose or some other electrophoretic medium that includes the ability to filter out some non-target molecules, is inserted into the sample tube 144 so that it is held just over an optional supporting mesh 149. The electrophoretic plug 146 can be introduced after the sample tube 144 is inserted into the capture tube 140, but it can be done before. As described above, the sample tube 144 can optionally have a supporting mesh 149 to help hold the plug in place and to facilitate removal. Alternatively, or in addition, the sample tube 144 can have ridges 148 or some other means for helping to hold the electrophoretic plug 146 inside sample tube 144. The sample tube 144 can be inserted into the capture tube 140 manually or with an automated picking and placing type of machine.

**[0098]** A sample 150 is then added over the electrophoretic plug 146, and a buffer 152 is added into the sample tube 144 over the sample 150. These steps could be done manually or in an automated manner. These combined tubes 140, 144 can then be collected into a tray or other holder that holds them together before or after the sample and buffer are added to enable electrodes 154 (only one is shown) to be inserted into the sample tube and moved below the bottom surface of the mesh support membrane 142 to electrophoretically cause the DNA in the sample to migrate. Alternatively, if a number of these individual assemblies were to be run in parallel on the same electrophoretic rig, then the assemblies with the electrophoretic plug, sample, and buffer would all sit in a holder such that a lower common buffer located on the

outside of each of the capture tubes rises above the height of the mesh support membrane with a single electrode submerged in a lower buffer chamber containing the lower common buffer.

**[0099]** The capture tubes can be held on a carousel and then transported along a conveyor belt. This system can thus allow processing in an in-line manner in which the sample tube is conveyed in a line and inserted into the capture tube with the mesh support membrane in an automated manner. Then the electrophoretic plug, sample, and buffer can be added. Alternatively, the electrophoretic plug is inserted into the capture tube prior to inserting the sample tube into the capture tube, and the sample and buffer are added after the sample tube is inserted into the capture tube.

**[00100]** Referring to FIG. 16, in another embodiment of the present invention similar to that shown in FIGS. 2-10, a volume extender system 160 is used rather than the volume extender plate 20 of FIG. 2. The system 160 has two volume extender units 162, 164, each of which can be substantially the same as the volume extender plate 20 of FIG. 2. Additionally, volume extending tubes 166 extend from the bottom of sample tubes 124 from the first volume extender unit 162 and into the top portions of sample tubes 125 of the second volume extender unit 164. The device shown in FIG. 16 can be used to provide additional volume for the sample compared to the volume extender plate 20 of FIG. 2.

**[00101]** Under electrophoresis, the sample and the buffer can undergo an osmotic expansion, such that the liquid expands upward as ions flow downward. The liquid in the tubes can thus expand upwardly into the well over the tubes, and potentially cause contamination between tubes. Consequently, it can be desirable to have additional volume to allow some expansion.

**[00102]** Some additional volume can be provided by extending the tubes of a volume extender plate of the type shown in FIG. 2. This can be accomplished to a point, but it is also desirable for the tubes to have good lateral stability at the top and at the bottom to make it easy to insert a

sample into the tubes and to ensure that the tubes nest within the capture plate easily. To address the desire for stability at both ends, the system shown in FIG. 16 provides such stability at each floor where the openings 126 are in each unit 162, 164 and from which the tubes extend.

**[00103]** In one embodiment, the volume of tubes from the first and second volume extender units 162, 164 and from the volume extending tubes 166 totals about 11 to about 12 ml for a process in which there is approximately 6 to 8 ml of sample and buffer combined. The difference between the total volume of the system and the volume of the sample and buffer is selected so that the osmotic expansion does not cause the liquid to overflow the top level of the tube.

**[00104]** In still another alternative embodiment, the walls surrounding the buffer well of the volume extender plate 20 of FIG. 2 can be made very small, or even reduced to substantially nothing so that the top portion of the volume extender plate is essentially a plate with openings and downwardly extending tubes. These tubes can be press fit into the plate for a integral construction, or the volume extender plate and the tubes can be molded as a single unitary and monolithic unit.

**[00105]** The following examples provide further details of the invention. Accordingly, while exemplified in the following manner, the invention is not so limited.

EXAMPLE 1

**[00106]** The following is an example of gel preparation, electrophoretic target capture, and target genomic DNA recovery. For the gel layer preparation, an amount of 29:1 acrylamide:bis-acryloyl cystamine (BAC) is mixed with capture probe of a given sequence, where the capture probe has a moiety capable of copolymerizing with the acrylamide and the polymerization is done in the presence of polyester mesh between glass plates. The resulting gel is formulated to

result in a 5% acrylamide gel with 20 μM of capture probe therein. Other embodiments of gel layer preparation are disclosed in Examples 2 and 3. After polymerization, the glass plates are removed and the gel is dried. The concentration of the capture probe may be varied, and the physical dimensions of the gel (i.e., diameter and thickness) may be varied to control ultimate binding capacity of the gel and DNA recovery.

**[00107]** Discs of desired diameter are punched out of the mesh and sealed to the bottom of the capture tubes prior to electrophoretic capture. Once sealed to the bottom of the capture tube, the mesh is rehydrated prior to sample introduction. Rehydration may be performed with any low salt concentration molecular biology buffer, such as $10^{-3}$ M Tris[hydroxymethlyl]aminomethane, $10^{-4}$ M ethylenediamine tetraacetic acid (TE) pH 8.0. The integrity of the gel layer and the seal should not allow flow paths around the gel. The dimensions of the tube are defined by the desired volume of sample to be processed.

**[00108]** The tube/gel assembly is then placed in the electrophoretic path of a sample containing nucleic acids complementary to the capture probes. The complementary nucleic acids hybridize to the capture probes during electrophoresis and are retained in the gel. Hybridization stringency may be modulated by a controlled temperature bath. Capture efficiency may also be controlled by voltage and total electrophoresis time. For target recovery, the disc is removed from the electrophoretic system and rinsed. Excess liquid is blotted or shaken off.

**[00109]** The gel is dissolved by adding 1.5 mM tris-carboxyethyl phosphine (TCEP) in 5 mM Tris[hydroxymethlyl]aminomethane (TRIS) pH 7.5 to 8.0. Using TCEP, the target nucleic acids and dissolved gel may be used with the polymerase chain reaction (PCR) described in Example 3. Other reducing agents, for example, dithiothreitol (DTT), are effective in dissolving the gel without inhibiting PCR.

## EXAMPLE 2

**[00110]** This Example is substantially the same as Example 1 above, except that the gel layer is prepared in such a way that the gel will not be dissolved in order to recover target molecules. Rather, the interaction between the capture probe and target is disrupted to release the target. Accordingly, instead of adding BAC as the crosslinker, an amount of a 40% 19:1 mixture of acrylamide/bisacrylamide is mixed with a capture probe of a given sequence. After electrophoresis is performed as in Example 3, rather than dissolving the gel, sodium hydroxide (NaOH), or alternatively heat, is introduced to disrupt the interaction between the probe and captured target molecule. Following disruption of the probe/target molecule interaction, recovery of the target molecule for further analysis or processing may be achieved by centrifugation, electrophoretic elution, or vacuum filtration.

## EXAMPLE 3

**[00111]** The following is an example of DNA capture and analysis using pre-cast membranes. It should be understood that this is an exemplary process for smaller-scale capture and analysis and that modifications would be made for higher volume processing.

### Preparation of Membranes

**[00112]** As a first step, the mesh support must be cleaned and sterilized. A mesh screen is prepared by cutting a Sefar 07-105/52 polyester mesh (Sefar America Inc., Mount Holly, NJ) into pieces of desired size, e.g., 3 inches by 4 inches (7.5 cm by 10 cm), or possibly a 10 inch (25 cm) square. The cut mesh pieces are placed in a plastic bag, sonicated for 10 minutes in 10% bleach, and rinsed two times with $H_2O$ before being sonicated for 10 minutes in 100% ethanol (EtOH). The sonicated mesh pieces are rinsed three times with reverse osmosis (ro) $H_2O$ and sonicated for 10 minutes in 1% sodium dodecylsulfate (SDS), dried, and stored until needed.

**[00113]** As a second step, glass plates are prepared. Using Kimwipes® absorbent wipes (Kimberly-Clarke Corp., Dallas, TX), glass plates are cleaned with water followed by EtOH. Then, the plates are sprayed with Rain-X® glass coating (Pennzoil-Quaker State Co., Houston, TX) which is distributed evenly with Kimwipe® absorbent wipes and allowed to dry. Excess Rain-X® glass coating is cleaned with EtOH and Kimwipe® absorbent wipes. The dried mesh is placed squarely in the center of a glass plate. Another glass plate is then placed onto the mesh such that the edge of the polyester mesh is even with the top of the plate. Alternatively, one of the glass plates completely covers the mesh while the other plate mostly covers the mesh except for a cut-out region at one end.

**[00114]** As a third step, a gel mixture is prepared. This gel formulation differs from previous examples insofar as ethyl glycol diacrylate (EGDA) is used as the crosslinker. Either of the gel layer formulations as described in Examples 1 and 2 can be substituted, however, the proper elution procedures should be maintained as described in each example. For preparation of the EGDA gel, the following reagents are mixed to prepare 1 ml of solution: 2.5 µl of EGDA; 22.5 µl of N, N' Dimethyl formamide, 119 µl of 40% acrylamide; 100 µl of 10X tris borate ethylenediamine tetraacetic acid (TBE), 20 µl of glycerol; 57 µl of 350µM Acrydite® -modified oligonucleotide (i.e., an acrylamide group is added to the 5' end of the oligonucleotide capture probe); 10 µl of fresh 10% ammonium persulfate; 1 µl of N-, N'-tetramethylenediamine (TEMED); and $H_2O$ to bring the total volume to 1 ml. Water and oligonucleotide volumes can be adjusted for use with oligonucleotides of other concentrations.

**[00115]** The mixture then is pipetted onto the top of the mesh enclosed by glass plates and the gel solution wicks into the pores of mesh. After the solution has been drawn into the mesh, a large binder clip is slowly applied to the top of the glass sandwich. Another large binder clip is applied to the bottom of the glass sandwich. The mixture is allowed to polymerize for

approximately one hour, forming a gel in which the capture probes are dispersed and copolymerized therein. After that time, the binder clips are removed and the glass plates are pried apart. The gel is suspended by clipping one corner of the polymerized gel with clips in a ventilation hood. The gel then is dried for approximately 12 hours and can be stored.

**Assembly of Capture Tube**

**[00116]** First, the support mesh membrane is bonded to a capture tube. Circles measuring 0.375 inches (0.94 cm) in diameter are punched out of the prepared dried mesh using a hole punch on a clean polyvinyl chloride (PVC) plate. Using forceps, the punched circles are collected into a small weighboat. The membrane circles carry a static charge, which can be at least partially discharged using a ZeroStat® antistatic gun (Structure Probe, Inc., West Chester, PA).

**[00117]** The membranes are fastened to capture tubes by using a welding device which is designed to match the diameter of the capture tube and melt the tube and capture membrane together, forming a water-tight seal in the process. The tip of welding device is cleaned with EtOH and Kimwipe® absorbent wipes. Polypropylene capture tubes having a 0.375 inch (0.94 cm) outer diameter and a 0.25 (0.61 cm) inch inner diameter are used. The tubes are placed onto a fixture of the welding device, which is set to approximately 240 degrees Celcius. Using forceps, a membrane circle is placed onto the capture tube. The tip of the welding device is brought in contact with the capture membrane and tube such that heat is transferred to the circumference of the tube in contact with the outer edge of the capture membrane for approximately 5 seconds. Immediately prior to using the capture device, the membrane is hydrated with sufficient 0.1X TE to cover its entire surface.

**[00118]** The sample tube/capture tube assembly then is prepared by connecting the sample tube and capture tube with a silicone tube sized to seal to the junction between both tubes. For

example, a 0.50 inch length of a 0.3 inch inner-diameter/ 0.56 inch outer-diameter silicone tube is placed onto the open end of a 0.375 inch outer-diameter/ 0.25 inch inner-diameter, 3 inch long sample tube. The capture tube is then inserted into the opposite end of the silicone tubing such that the silicone tube is form fitted to the capture tube to prevent sample leakage.

**[00119]** To prepare an agarose plug, a solution of 1% SeaKem® LE agarose (Cambrex Corporation, East Rutherford, NJ) in 1X TBE is prepared in a 60 degree Celsius water bath. Using a narrow-bore transfer pipette, the capture tube is filled with molten agarose up to the top. The membrane and agarose are checked for the presence of air bubbles. Different preparations of agarose can be used to vary the relative porosity and thus vary the filtration properties of the agarose plug. The capture tube assembly then is inserted into holes in an electrophoresis unit.

**Electrophoresis Setup**

**[00120]** Up to six assembled capture tubes are seated into the holes of a Bio-Rad® (Bio-Rad Laboratories, Inc., Hercules, CA) Model 422 electro-eluter. The unit is labeled with sample identities and placed into the tank. A blank membrane is included for each specific capture. The tank is filled with 1X TBE running buffer so that the bottoms of the capture tubes are submerged. If 3 inch tubes are used, the lower electrode is submerged.

**Sample Preparation**

**[00121]** A crude stool sample is spun for at least 15 minutes at 14,000 rpm in a microfuge to remove any particulates. For each 100 µl of stool, an amount of 41.7 µl formamide, 16.7 µl of 890 mM TBE, and 8.3 µl of 0.025% bromophenol blue and xylene cyanol (BB/XC) is added. The sample at this point is composed of 89 mM TBE, 25% formamide, and 0.005% each BB/XC. This amount is pipetted repeatedly to mix. A Costar® 0.8 µm filter (Corning Costar Corp., Cambridge, MA) is affixed to a 3.0 ml syringe. The entire sample is transferred to the syringe and filtered into a Falcon® tube (Becton, Dickenson, and Co. Corp., Franklin Lakes, NJ).

The DNA in the samples are then denatured by placing the tubes in a boiling water bath for 10 minutes.

**[00122]** The sample is then iced for 5 minutes and up to 2 ml of the denatured sample is pipetted into the capture tube assembly. The tube is topped off with 1X TBE (running buffer) and then the upper buffer reservoir is also filled with 1X TBE so that it covers the tops of all of the sample tubes. The cover on the unit is placed observing the polarity of the contacts and connected to the power supply.

**Electrophoresis**

**[00123]** The sample is electrophoresed for 16 hours at 15V in the downward direction, such that the lower buffer chamber contains the anode and the upper buffer chamber contains the cathode. The buffer is then poured off. The capture tube assemblies are removed and disassembled to separate the sample tubes from the capture tubes. The agarose plugs then are removed from the capture tubes. The capture tubes are rinsed with fresh TBE and fitted into an electro-eluter with fresh buffer in the lower chamber such that the lower end of the tubes are submerged in the buffer. The capture tubes are then filled with fresh buffer. The polarity of the contacts is reversed such that the anode is now in the top buffer chamber and the cathode in the lower chamber. The sample is electrophoresed for 3 hours at 30 V. The buffer is then poured off and the capture tubes are rinsed with 150mM NaCl and 15 mM TRIS pH 7.5.

**[00124]** The capture tubes are then rinsed with 0.1X TE. Each capture tube is placed, membrane down, into a labeled 1.5 ml Eppendorf® tube (Eppendorf Co., Hamburg, Germany) and 10 µl of 0.1N NaOH (elution buffer) is added on top of the membrane. The elution buffer is allowed to sit in contact with the membrane for approximately 10 to 15 minutes at room temperature in order to dissolve the gel. The tubes with the membrane are then briefly spun in a microfuge. The tubes are left in the microfuge, and 10 µl of 0.1N HCl is added. Then, the tubes

are briefly spun again. Next, the tubes are left in the microfuge, and 30 µl of 1X TE is added. The tubes are briefly spun again. The samples are then removed from the microfuge, and the capture tubes are discarded. The samples are stored until further analysis at 4 degrees Celsius if analyzed within 8 hours or at -20 degrees Celsius if analyzed at any time longer than 8 hours from recovery.

**Analysis of Sample (First Example of Analysis)**

**[00125]** Fifty microliters of a sample is transferred into column 1 of a round-bottom 96-well plate. The blank sample is transferred into column 7. Next, 40 µl of (ro)$H_2O$ is pipetted into columns 2 through 6 and column 8. An amount of 20 µl is serially diluted from column 1 through row 6 for the specific captures and from column 7 into column 8 for the blanks. Five microliters of dilutions 1 through 4 of the specific captures and dilution 1 of the blanks is used for TaqMan® (Roche Molecular Systems, Inc., Branchburg, NJ) analysis specific for the capture sequence. Five microliters of all dilutions is used for LATaq endpoint PCR. PCR is performed with primers specific for the capture sequence. Five microliters of dilutions 1 and 2 of both specific captures is used as well as blanks for LATaq endpoint PCR with non-specific primers.

**Analysis of Sample (Second Example of Analysis)**

**[00126]** Sample analysis may be performed to demonstrate sample compatibility with PCR using a standard PCR multiple mutation protocol, or alternatively, a quantitative PCR protocol may be used to demonstrate recovery. Any of the sample analysis protocols may be performed on the previous examples as well as the forthcoming examples.

**[00127]** In one multiple mutation protocol, PCR is set up in a Misonix® (Misonix Inc., Farmingdale, NY) PCR hood using a premix solution of: 19.5 µl sterile molecular biology grade distilled $H_2O$, 5 µl of 10X LA PCR buffer II (Takara.Mirus.Bio Company), 10 µl of 2 mM 10X dNTP's, 5 µl of 10X PCR primers, and 0.5 µl LATaq polymerase, for a total volume of 40 µl.

The PCR reaction mix is composed of 40 µl of the premix solution plus 10 µl of the sample DNA or control. All PCR reactions are next run in a MJ Research® (MJ Research Inc., Waltham, MA) Tetrad Thermocycler overnight and immediately run on agarose gel or stored at -20 degrees Celsius. The samples are then analyzed for mutations.

**Analysis of Sample (Third Example of Analysis)**

**[00128]** In the quantitative PCR protocol, the captured stool DNA samples are amplified using Real Time PCR with a PCR primer set, TaqMan® probe, and Bio-Rad's iCycler iQ® Real Time PCR Detection System (Bio-Rad® Laboratories, Inc., Hercules, CA) . Quantitation is achieved by measuring an increase in fluorescence during the exponential phase of PCR.

**[00129]** The premix solution is prepared by combining 24.75 µl of sterile molecular biology grade distilled $H_2O$, 5 µl of 10X LATaq buffer II (Takara.Mirus.Bio Company), 10 µl of 2 mM dNTP's, 5 µl of 10X LcDIA primers/probe, and 0.25 µl of 5µ/µl LATaq , for a total volume of 45 µl. The premix solution is added to each well of a 96 well plate, and then 5 µl of sample is added to the desired wells. After addition of sterile water to blank wells for negative controls and addition of 5 µl of LcDIA human genome (HG) DNA standard to each of four wells, the 96 well plate is centrifuged at 1800-2200 RPM momentarily. The plate is then placed into the pre-heated iCycler® System and the software is run per Bio-Rad instructions in order to analyze the samples.

EXAMPLE 4

**[00130]** This example is substantially similar to Example 3, except that multiple layers of electrophoretic medium containing capture probes are sequentially added to the capture tube assembly after the supporting mesh with copolymerized capture probes is welded to the bottom of the capture tube.

## EXAMPLE 5

[00131] This example is similar to Example 3, except that multiple samples are processed using a device similar to that described in Figures 2-10.

**Sample Preparation**

[00132] A frozen stool sample with a total volume of 9.6 ml is thawed at room temperature for 30-45 minutes and centrifuged for 15 minutes at 16,500 x gravity (g). The supernatant is filtered through a 0.8 micron cellulose acetate syringe filter, and 1X TE is added to bring the volume to 10 ml. Next, 5.4 ml of load buffer (3.86 ml formamide and 1.54 ml of 10X TBE) is added to each sample tube. Samples are vortexed, incubated for 15 minutes in a 95 degree Celsius water bath, and placed on ice for 15-30 minutes prior to loading.

**Preparation of Membranes**

[00133] The membrane is prepared and welded to the bottom of each capture tube of a capture plate as described in Example 3. Each membrane is hydrated for 1 hour with 250 µl of 0.1X TE immediately prior to addition of the sample.

**Attachment of Volume Extender Plate to Capture Plate**

[00134] Six hundred microliters of 1% SeaKem® (FMC Corp., Philadelphia, PE) agarose is added to each capture tube of the 48 capture tube capture plate. Next, a volume extender plate is engaged to the capture plate by holding the volume extender plate at an angle while sequentially lining up the rows of openings of the two plates. When all of the rows of the volume extender plate and capture plate are in alignment, gentle pressure is applied to the plates ensuring that the volume extender plate is completely nested in the capture plate. The agarose is then allowed to set for 15 minutes.

**Electrophoresis**

[00135] The lower buffer chamber of an electrophoresis unit is filled with 1X TBE, and the capture plate assembly is placed in the unit at an angle such that no air bubbles form beneath the

tubes of the capture plate. The sample is then loaded equally into the tubes, and the remaining space at the top of the each tube of the volume extender plate is topped off with 1X TBE. In this example, 10 samples are each divided between 4 tubes, and 8 tubes are used as controls. The upper 48 channel electrode assembly is inserted into the volume extender plate/capture plate assembly such that each tube has an electrode contacting the TBE buffer on top of the sample. The samples are electrophoresed for 16 hours at 23 V, with a desirable current of 50-60 mA.

**Sample Recovery**

**[00136]** Once electrophoresis is complete, the electrophoresis unit is disassembled and the volume extender plate is removed from the capture plate, ensuring that the agarose is fully removed from capture tubes of the capture plate. The volume extender plate may be cleaned and reused or may be discarded. Each tube of the capture plate is rinsed twice with 200 µl of sodium TRIS (ST) buffer (150 mM NaCl and 15 mM Tris, pH 7.4), and the capture plate is reinserted into a reverse electrophoresis unit (i.e., an electrophoresis unit having a polarity opposite from the electrophoresis unit used to perform the first electrophoresis step). Fresh 1X TBE is placed in the lower buffer chamber and the top of the capture plate, ensuring that the levels of TBE inside the tubes of the capture plate and within the buffer chamber are equal. Reverse electrophoresis is performed for 3 hours at 27 V, with a current between 170-200 mA.

**[00137]** After reverse electrophoresis, the capture plate is removed from the reverse electrophoresis unit, inverted, and centrifuged in an inverted position for 2 minutes at 1,900 x g to remove any remaining buffer liquid. The capture plate then is nested with the membrane side down into a sample collection plate. Next, 44 µl of .1 N NaOH (elution buffer) is added into each well to dissolve the gel, and the capture plate/collection plate complex is covered and rocked at room temperature for 30 minutes. The plates are then centrifuged for 7 minutes at 1,900 x g.

**Analysis**

**[00138]** Analysis of samples can be performed by at least any of the enumerated PCR methods described in Example 3; however, this example utilizes a standard multiple mutation PCR analysis as described in Example 3 and the samples are prepared as follows. Neutralization buffer is prepared by mixing 4.28 ml molecular biology grade water, 5 ml of 1 N HCl, 0.6 ml 1 M Tris (pH 9.0), and 120 µl 500 mM EDTA, final pH adjusted to 8.8 - 9.5. A 96 well round bottom plate (the storage plate) is prepared, and 32 µl of neutralization buffer is placed into each of the 48 wells.

**[00139]** The collection plate is then separated from the capture plate, and the four wells corresponding to each stool sample are pooled and placed into one well of the storage plate. In this experiment, eight wells of the storage plate are controls, and up to forty wells may receive sample. The samples are then stored at -20 degrees Celsius until PCR analysis.

**[00140]** While the captured nucleic acids can be used for different tests, one use is to detect colorectal cancer from stool samples. Various screening assays can be used, including enumerated LOH, a DNA integrity assay, mutation detection, expression assays, and FISH.

**[00141]** Having described certain embodiments of the invention, it will be apparent to those of ordinary skill in the art that other embodiments incorporating the concepts disclosed herein be used without departing from the spirit and scope of the invention. The described embodiments are to be considered in all aspects as only illustrative and not restrictive.

**[00142]** What is claimed is:

## CLAIMS

1. A device for recovering a target molecule from a biological sample, the device comprising:

a first unit defining a chamber, the chamber comprising a first region for receiving a sample and a second region comprising at least one electrophoretic medium; and

a second unit extending from a first end to a second end and interfaceable with the first unit, wherein a mesh structure impregnated with an electrophoretic medium comprising capture probes copolymerized thereto is located at the second end of the second unit and wherein the second region is located between the first region and the mesh structure when the first unit is interfaced with the second unit.

2. The device of claim 1 wherein the first unit comprises a tube.

3. The device of claim 1 wherein the first unit further comprises a support at an end of the first unit.

4. The device of claim 3 wherein the support comprises at least one of a ridge, a solid support, and a mesh.

5. The device of claim 1 wherein the electrophoretic medium of the first unit and the electrophoretic medium of the second unit comprise the same material.

6. The device of claim 1 wherein the electrophoretic medium of the first unit and the electrophoretic medium of the second unit comprise a different material.

7. The device of claim 1 wherein the first unit further comprises one or more additional electrophoretic media.

8. The device of claim 1 comprising a plurality of first units and a plurality of second units.

9. The device of claim 1 wherein the first unit is capable of nesting within the second unit.

10. The device of claim 1 wherein the mesh structure is attached to the second unit at the second end.

11. The device of claim 1 wherein the mesh structure comprises a non-conductive material.

12. The device of claim 1 wherein the electrophoretic medium of the second unit comprises a plurality of identical capture probes.

13. The device of claim 1 wherein the electrophoretic medium of the second unit comprises a plurality of different capture probes.

14. The device of claim 1 wherein the capture probes are substantially evenly dispersed within the electrophoretic medium of the second unit.

15. The device of claim 1 further comprising a third unit interfaceable with the second unit, wherein the third unit defines a well.

16. The device of claim 15 wherein the second unit is capable of nesting within the third unit.

17. A device comprising:

a housing defining a chamber, the chamber comprising a first region for receiving a sample and a second region comprising at least one electrophoretic medium; and

a mesh structure impregnated with an electrophoretic medium comprising capture probes copolymerized thereto, wherein the second region is located between the first region and the mesh structure and the mesh structure is located at an end of the device.

18. The device of claim 17 further comprising a third unit defining a well, the unit being interfaceable with the end of the device.

19. The device of claim 17 wherein the electrophoretic medium of the second region and the electrophoretic medium of the mesh structure comprise the same material.

20. The device of claim 17 wherein the electrophoretic medium of the second region and the electrophoretic medium of the mesh structure comprise a different material.

21. A method for recovering a target molecule from a sample, the method comprising:

applying a vacuum to a sample comprising a target molecule to move the sample from a first area to a second area; and

applying an electric motive force to the sample to move at least the target molecule in a first direction such that the target molecule interacts with a capture probe copolymerized to an electrophoretic medium.

22. The method of claim 21 further comprising releasing the target molecule from the capture probe and applying a second electric motive force to move the target molecule in a second, different direction.

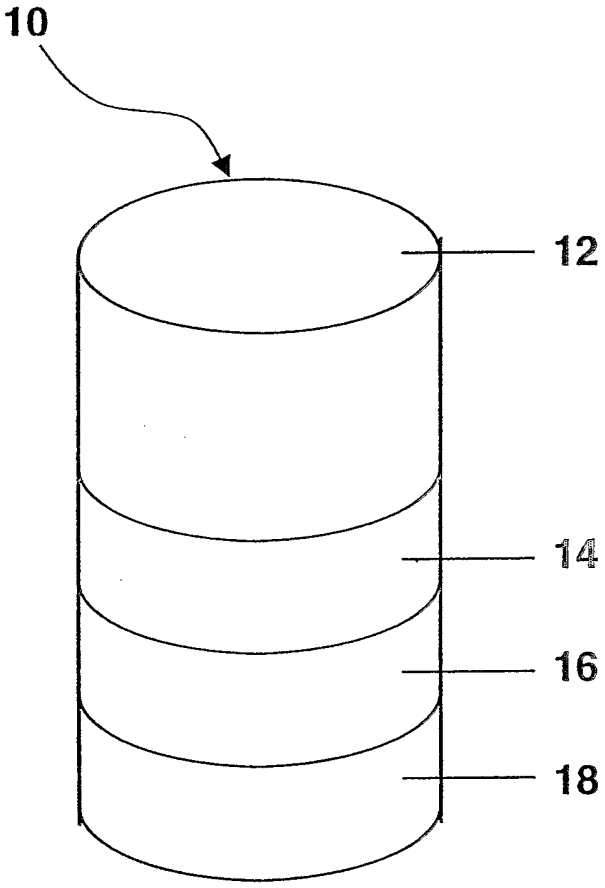


FIG. 1

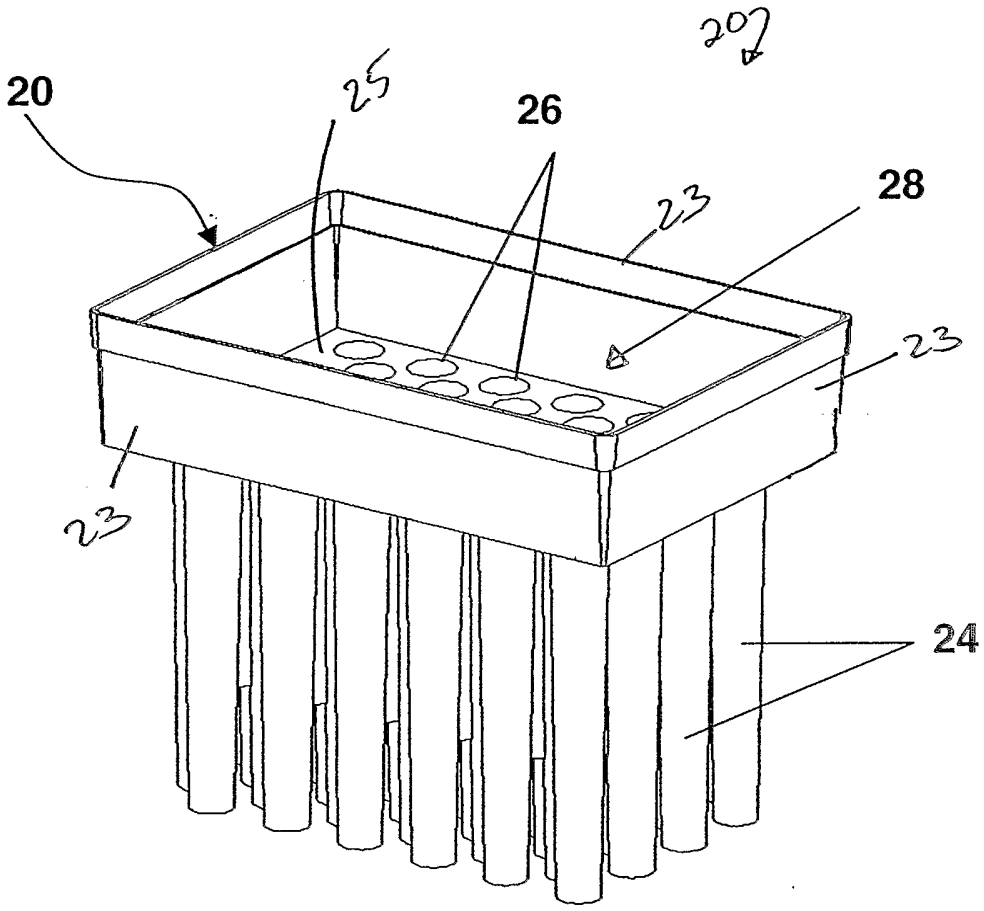


FIG. 2

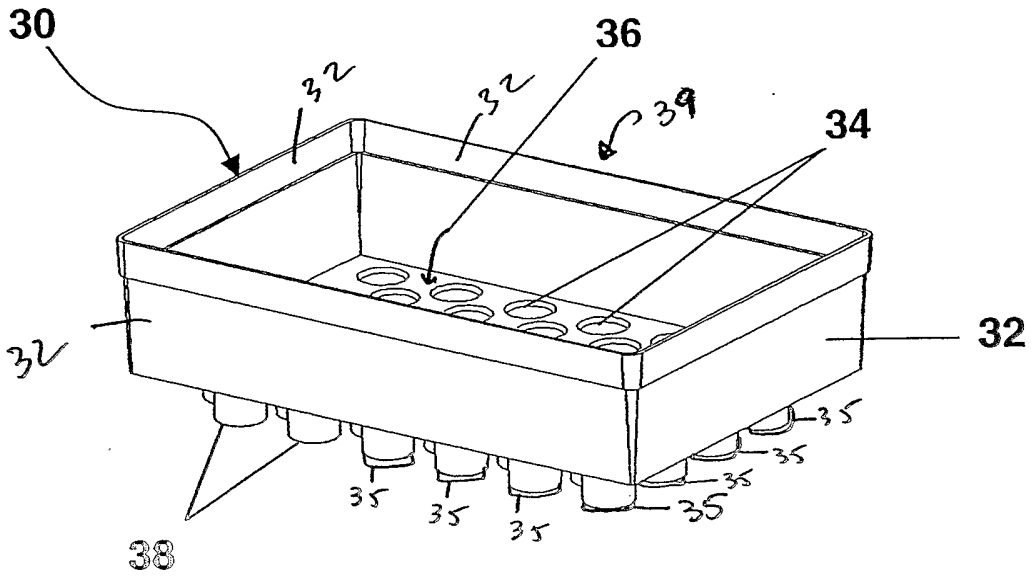


FIG. 3

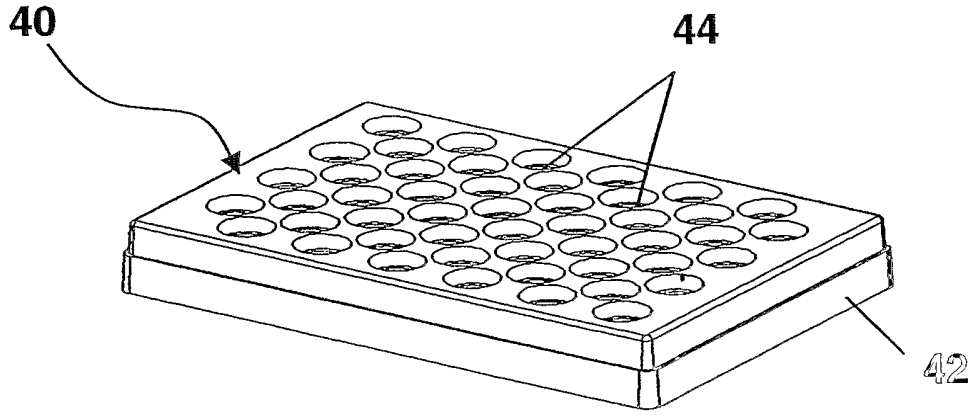


FIG. 4

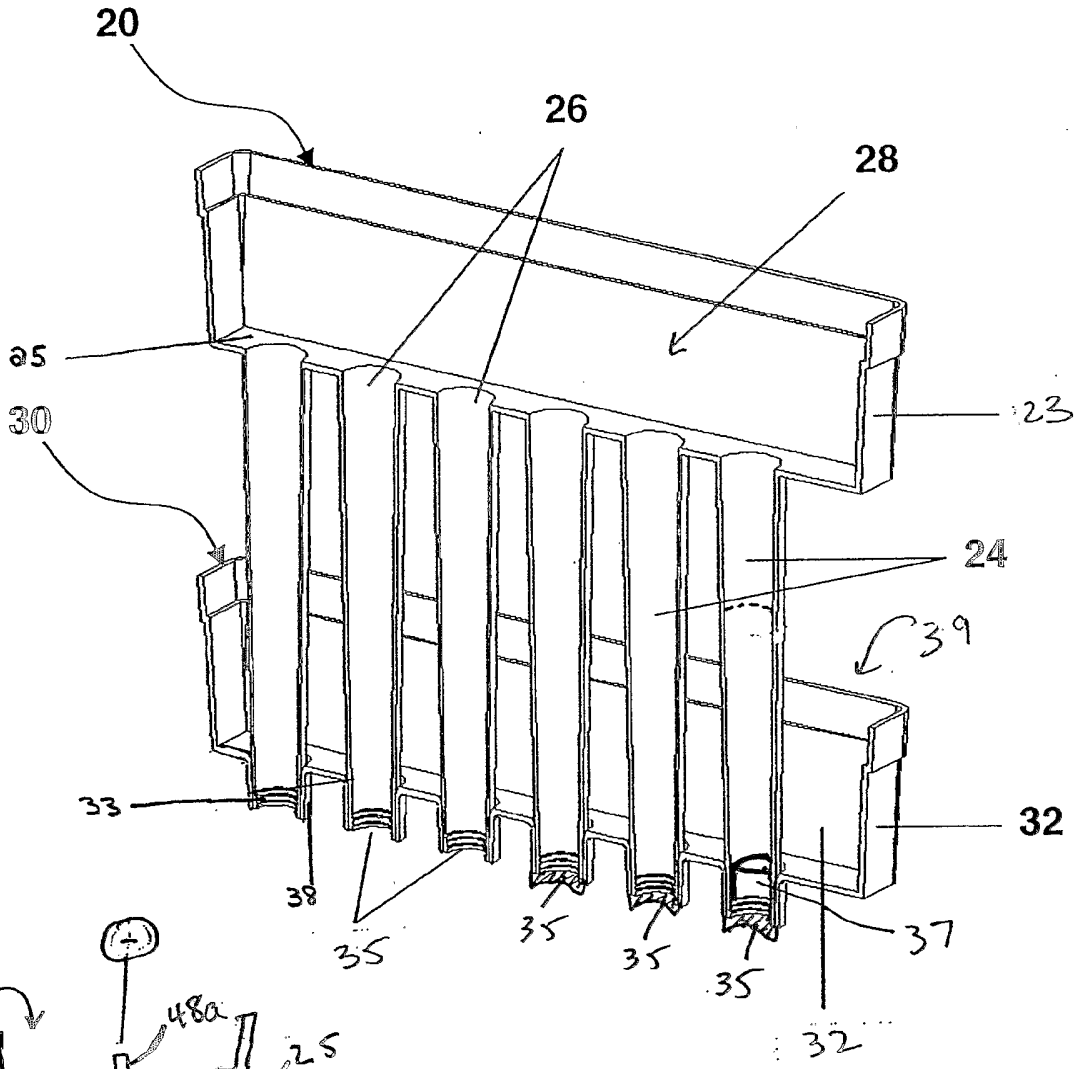
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FIG. 5

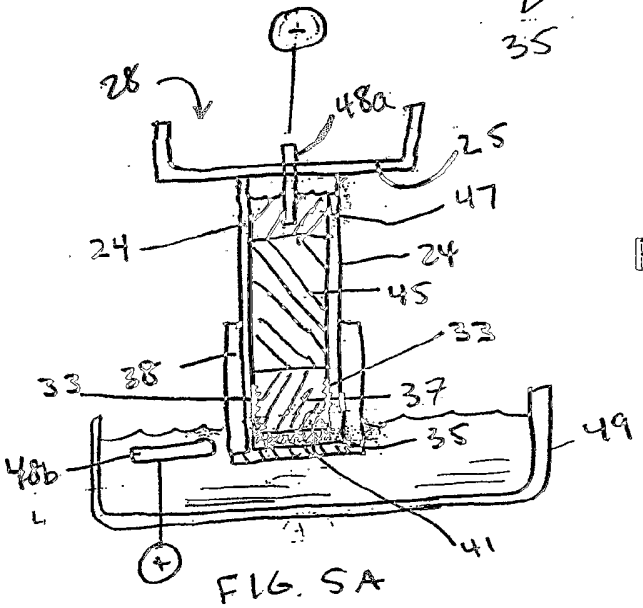
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FIG. 5A

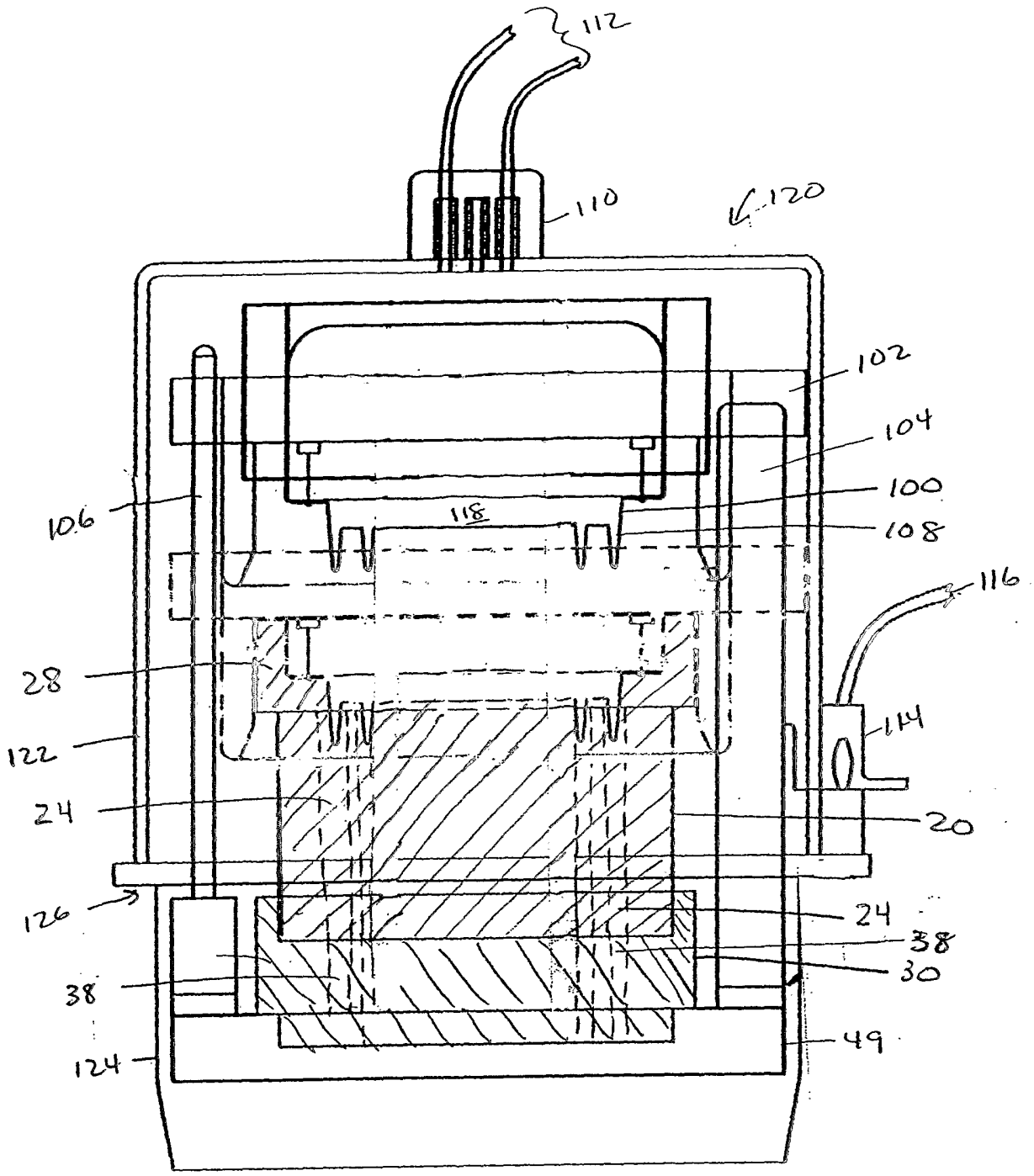
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FIG. 5B

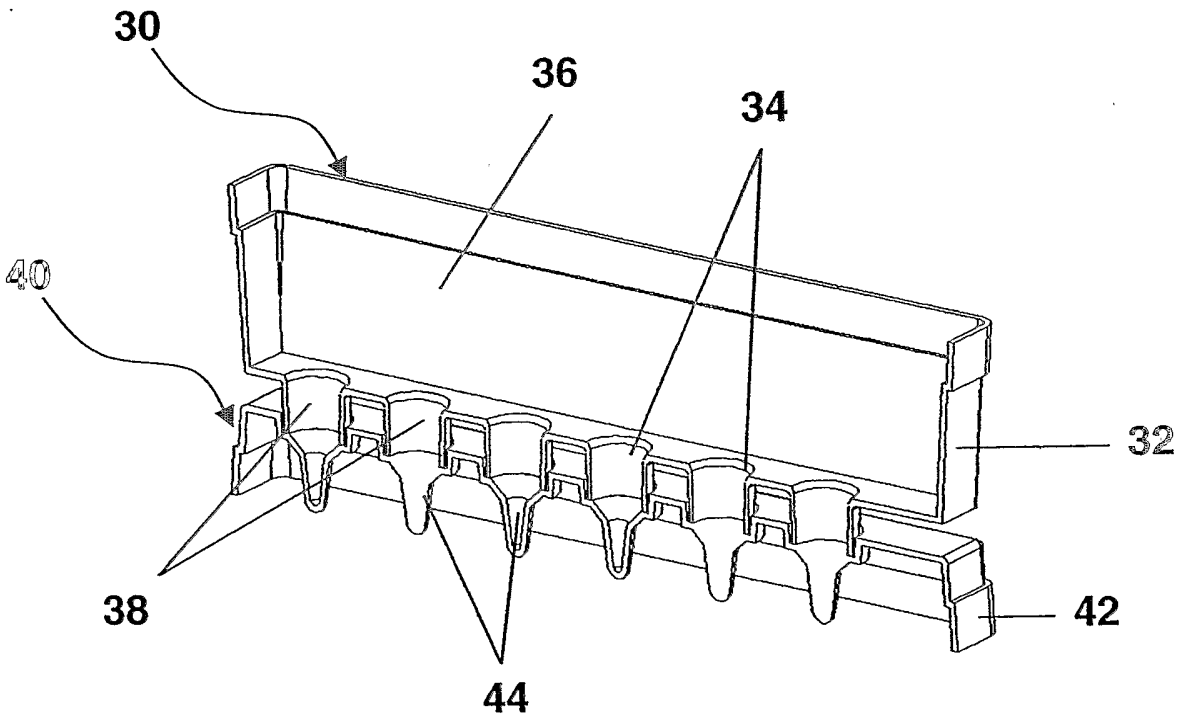


FIG. 6

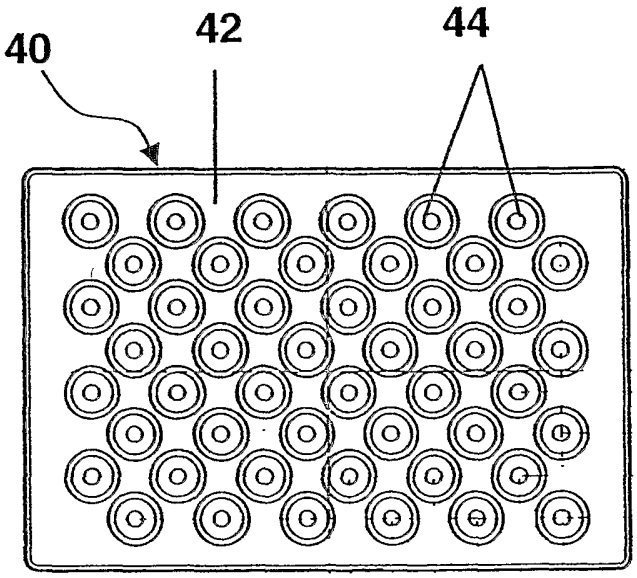


FIG. 7a

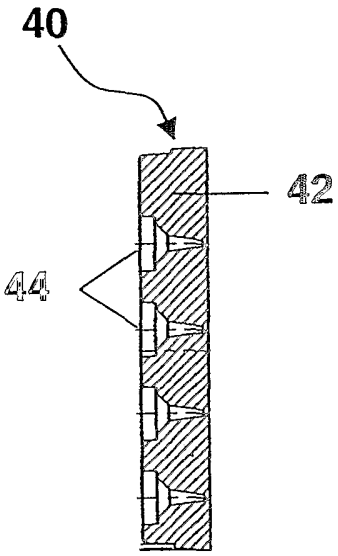


FIG. 7c

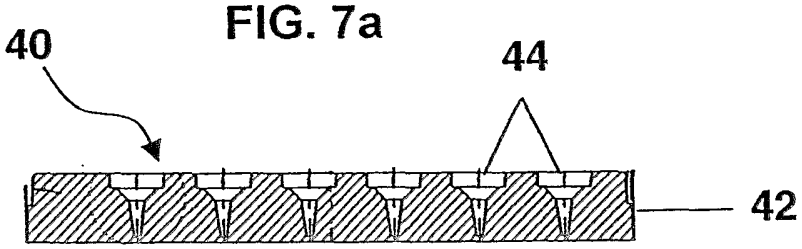


FIG. 7b

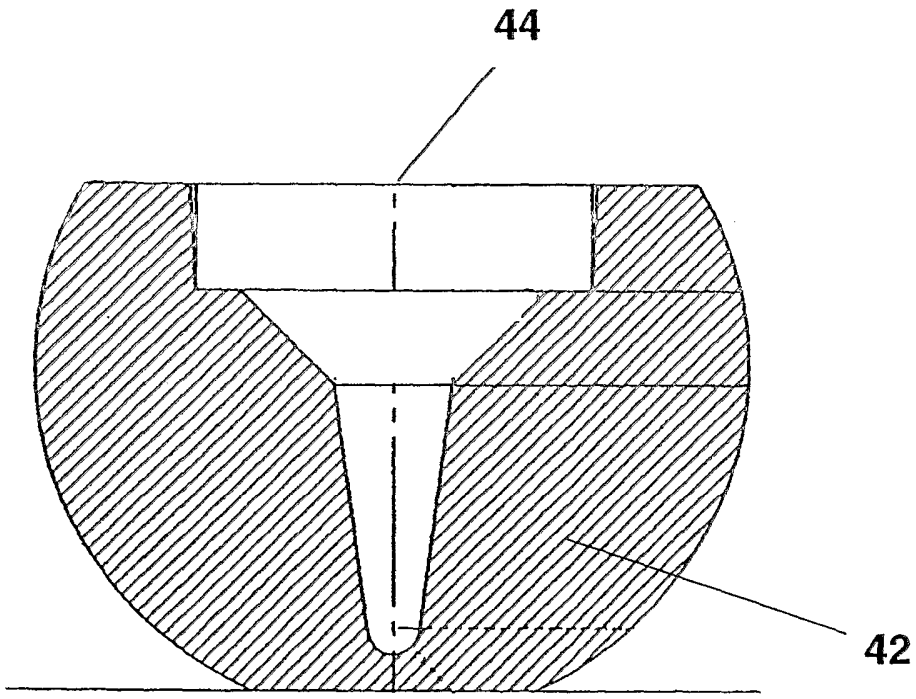


FIG. 7d

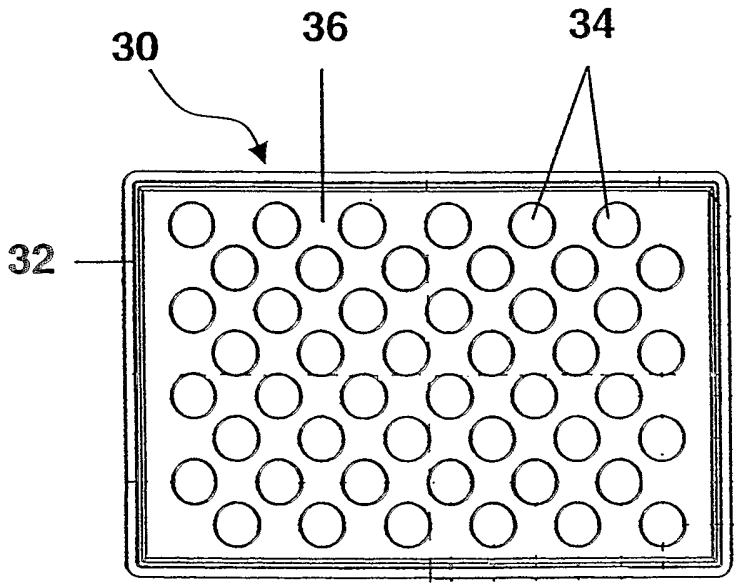


FIG. 8a

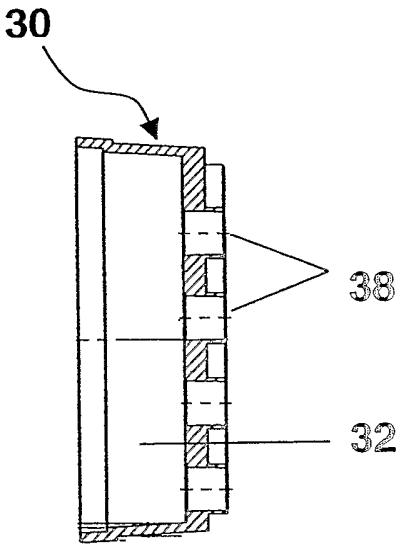


FIG. 8c

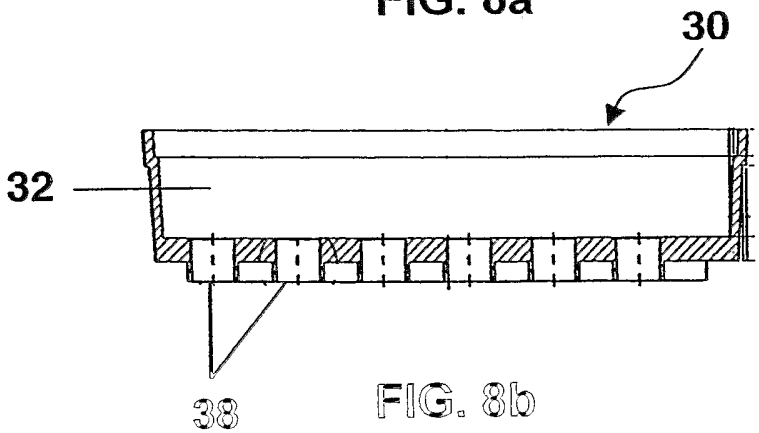


FIG. 8b

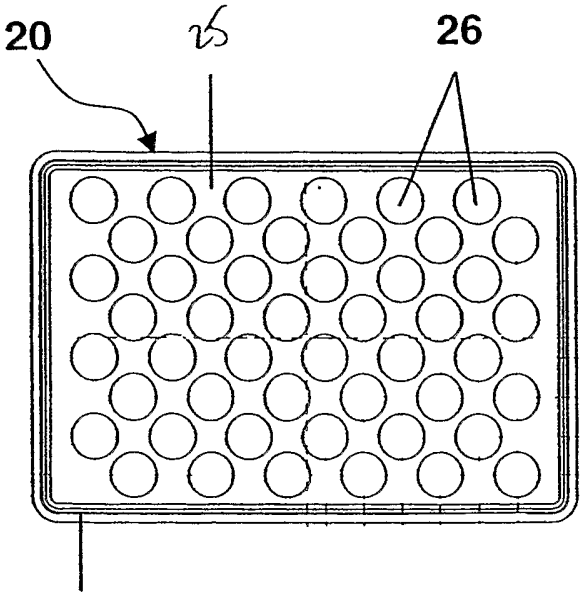


FIG. 9a

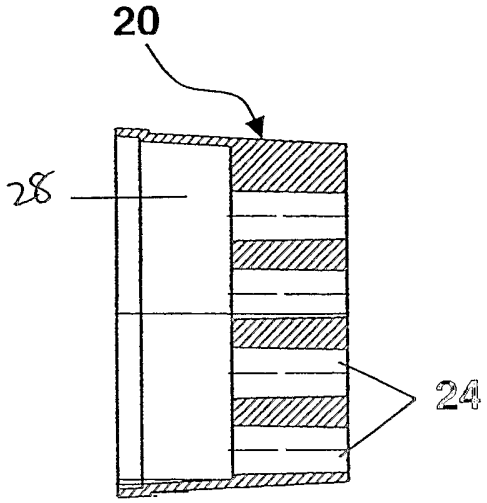


FIG. 9c

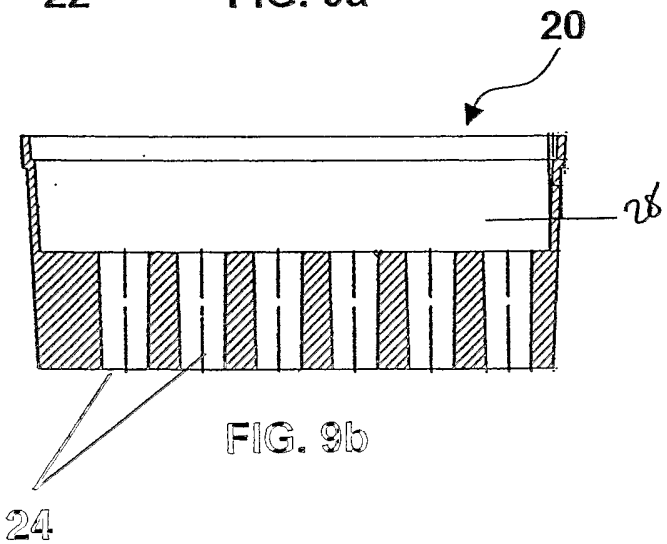


FIG. 9b

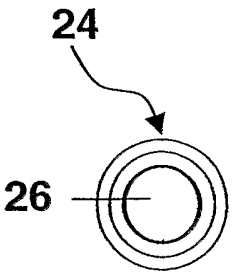


FIG. 10a

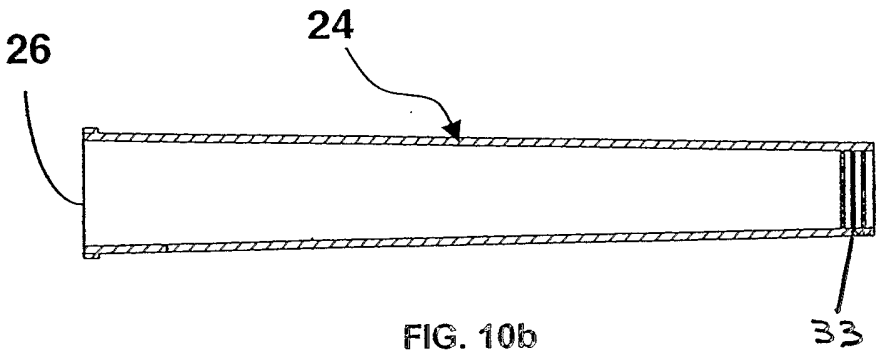


FIG. 10b

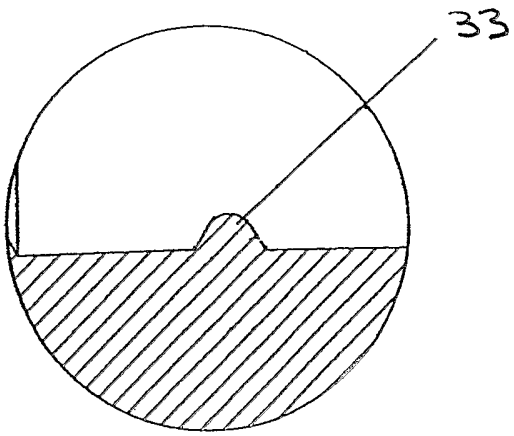


FIG. 10c

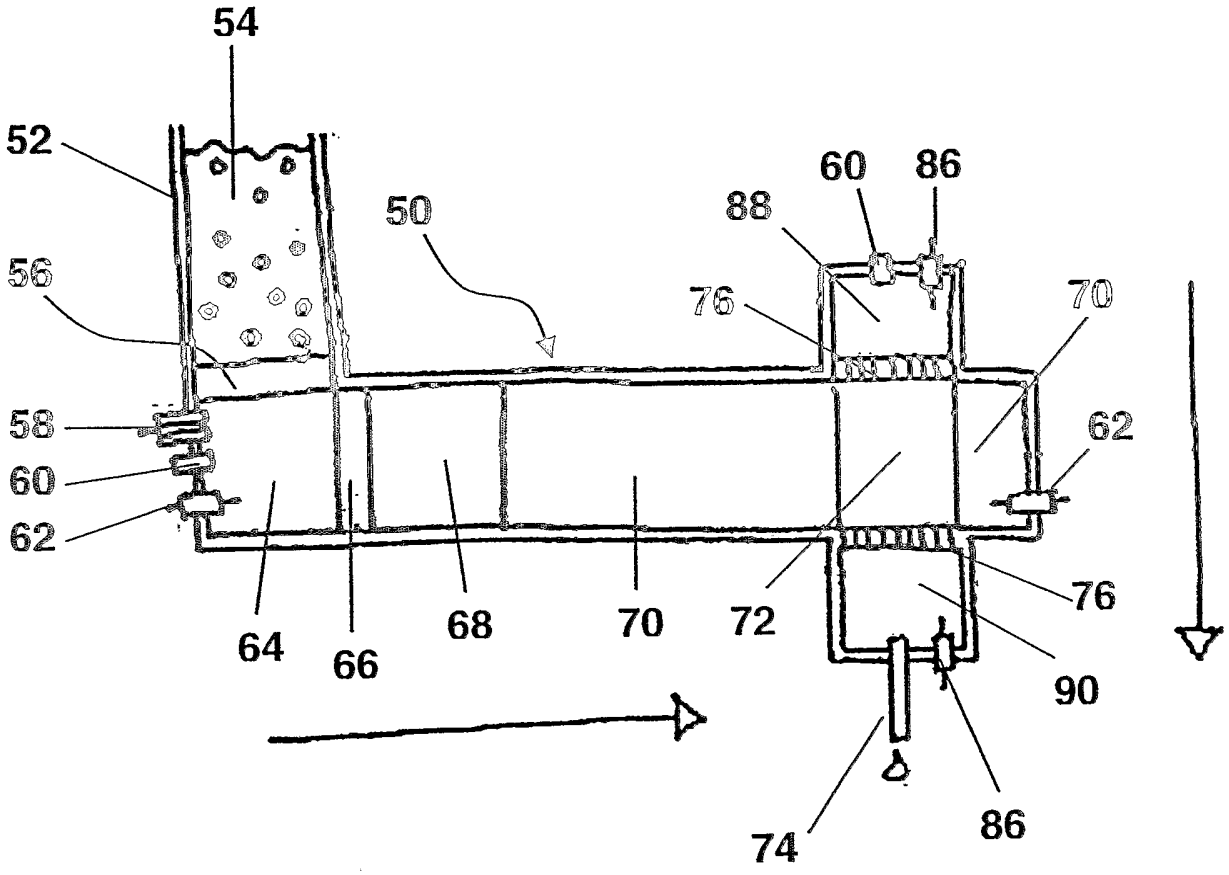


FIG. 11

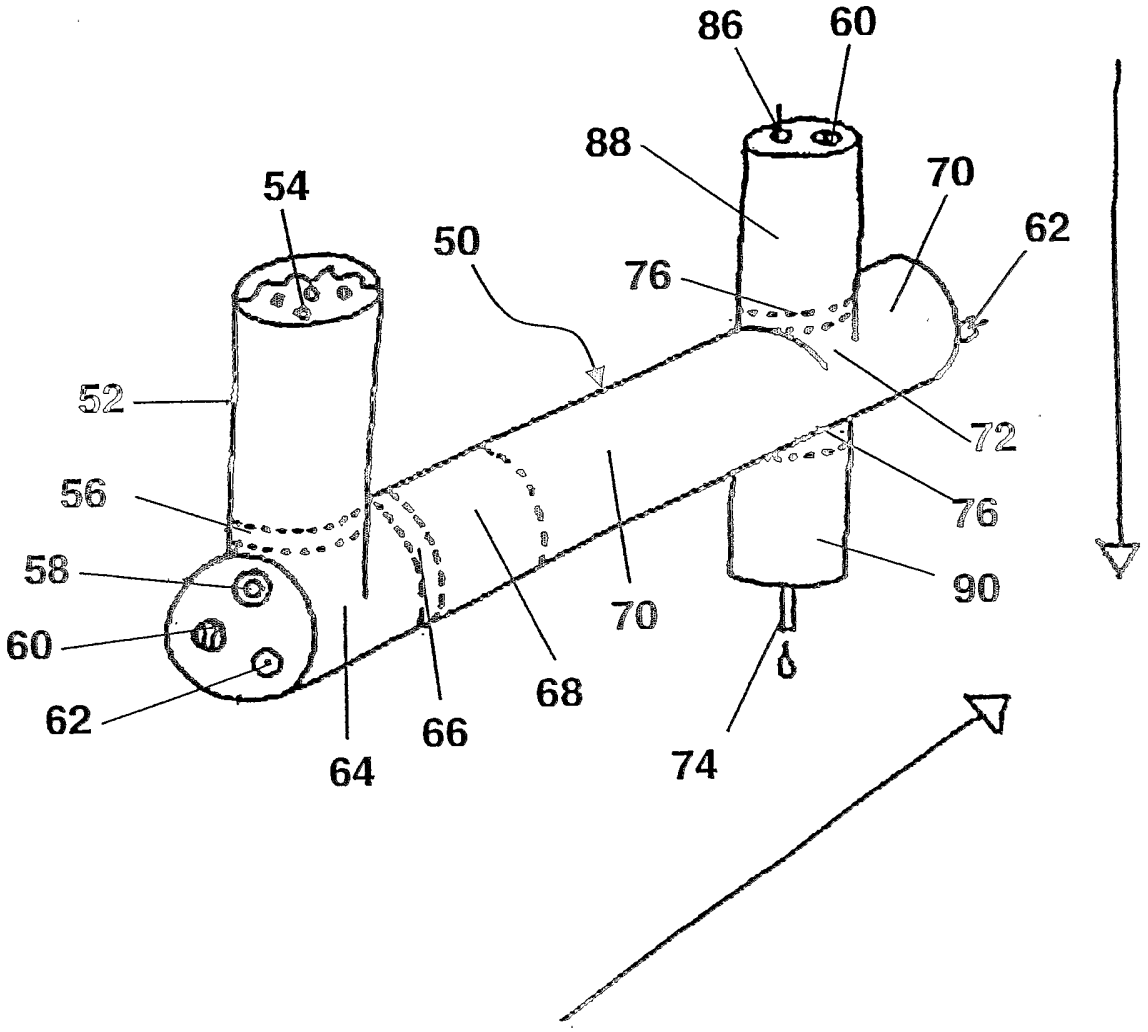


FIG. 12

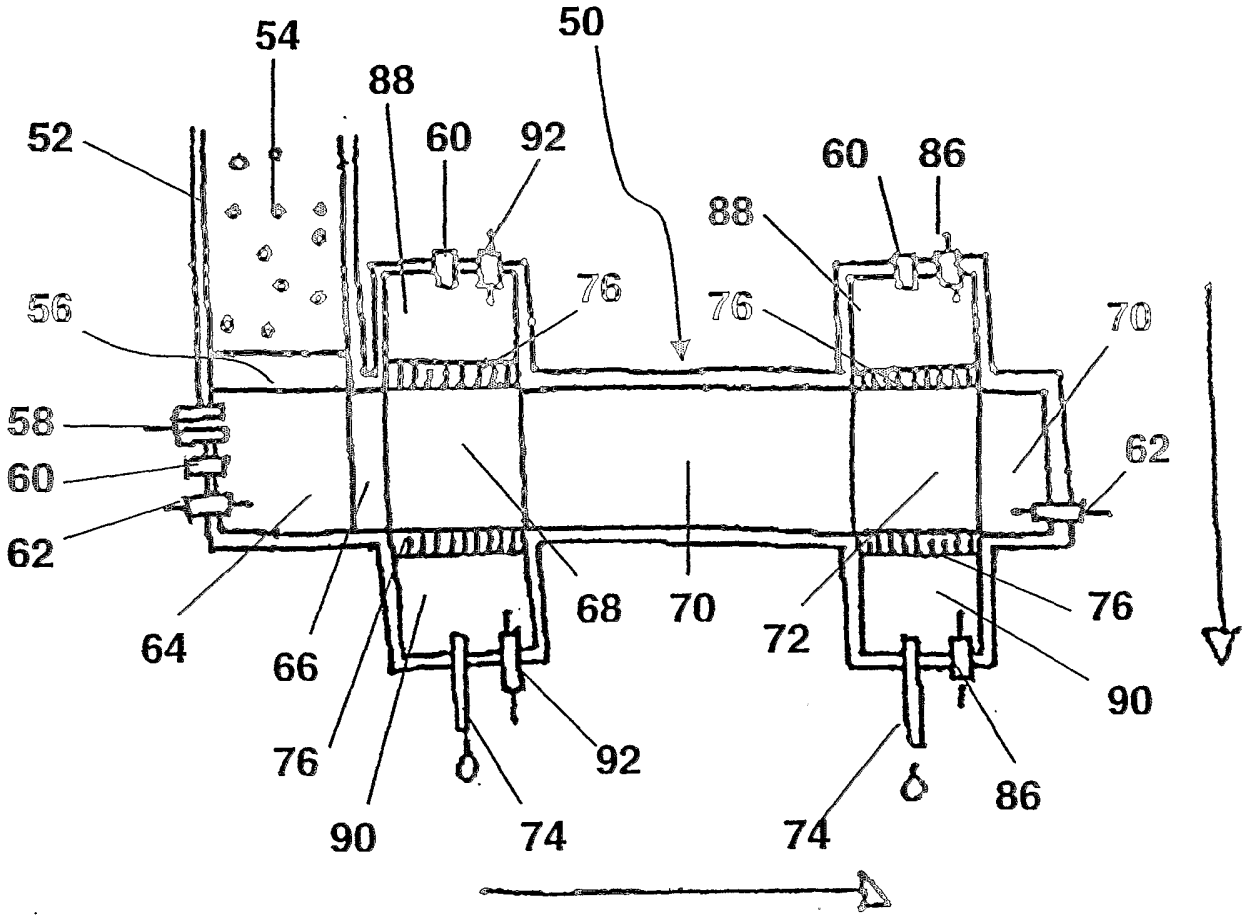
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FIG. 13

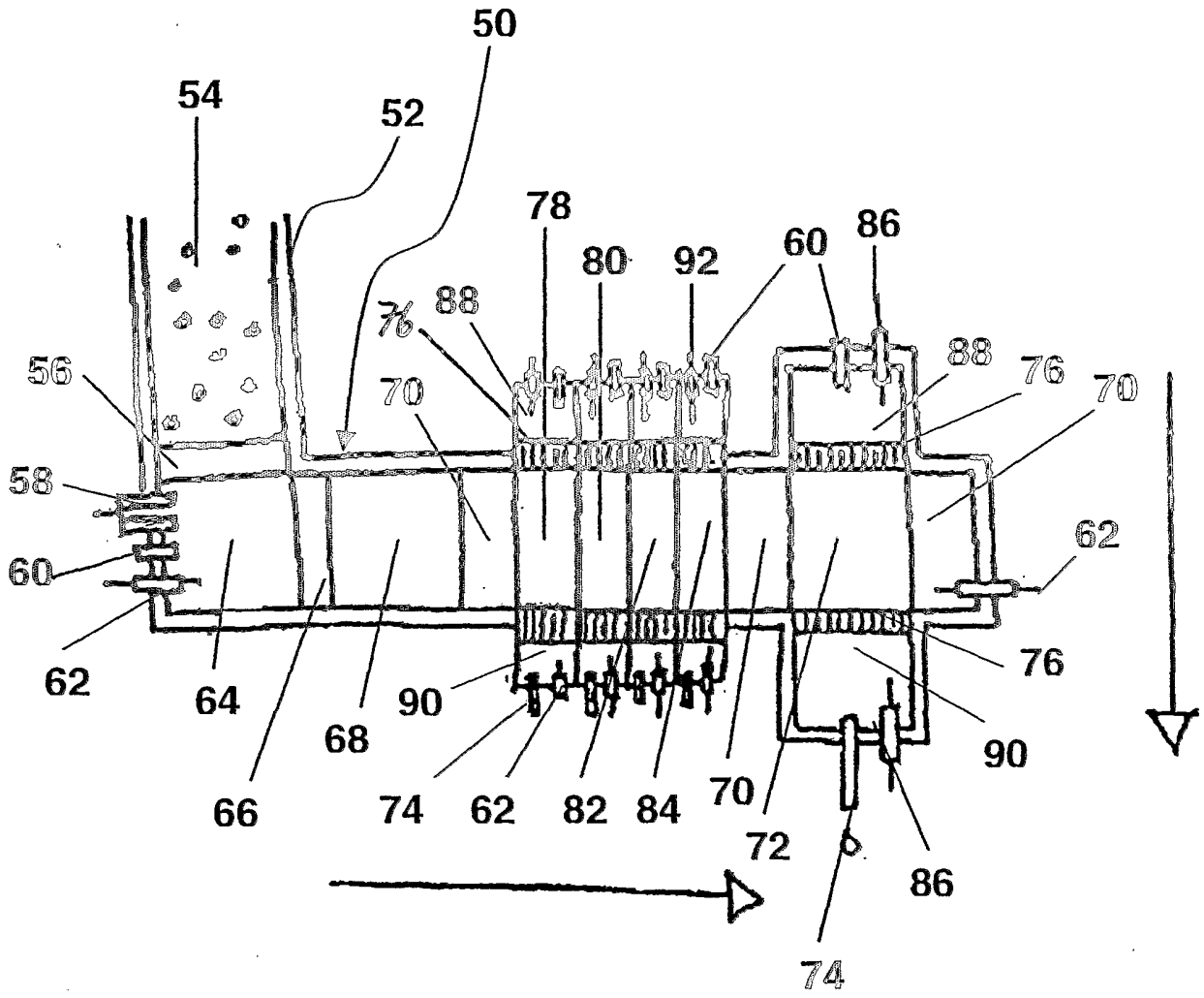
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FIG. 14

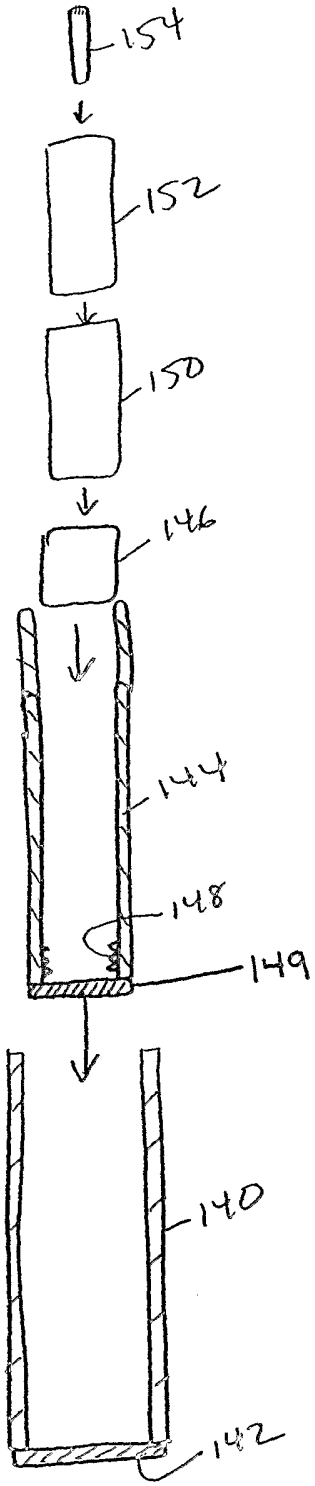


FIG. 15

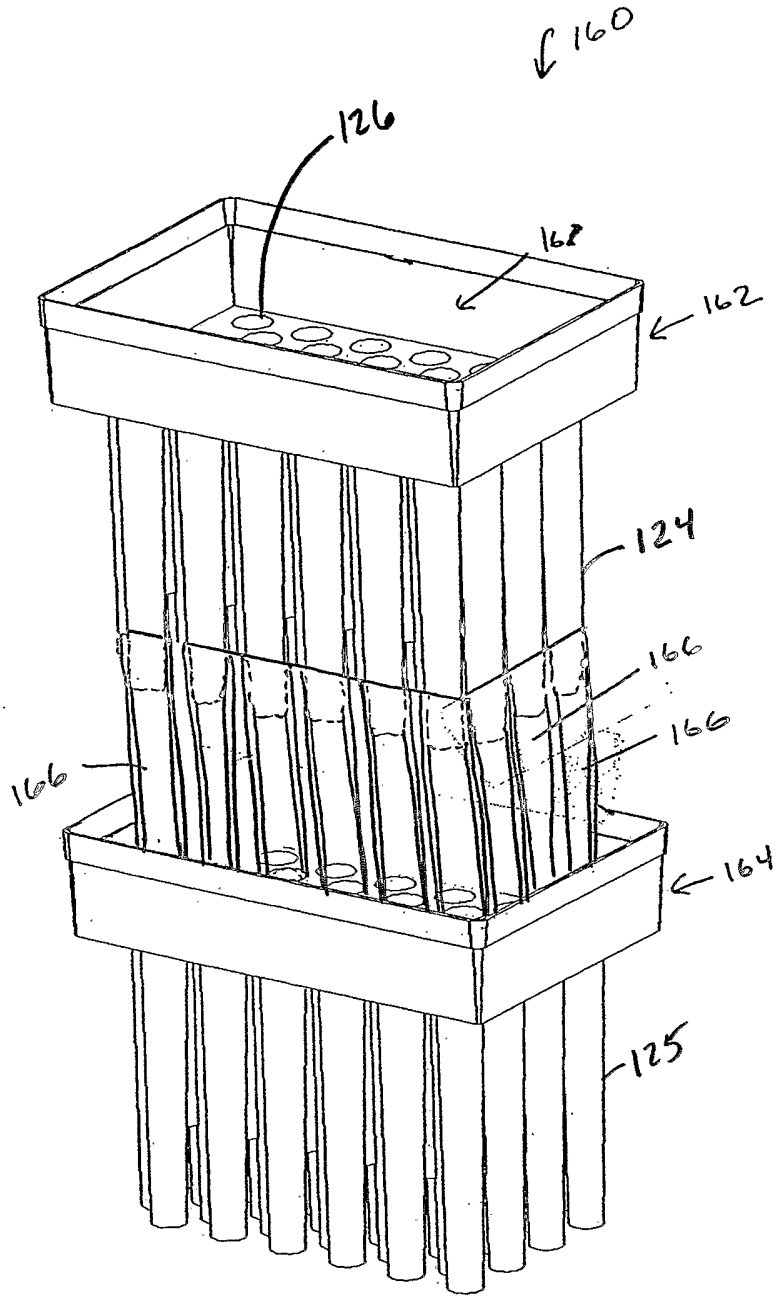


FIG. 16